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A redefinition of *Pseudokellya* Pelseneer, 1903 (Bivalvia: Cyamiidae) and the description of a new species from the Southern Ocean

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ABSTRACT

A new species of *Pseudokellya*, *Pseudokellya franki* from South Shetland Islands, is described. *P. franki* is characterized by its subcircular shell outline, the periostracum forming low lamellate commarginal folds, radial sculpture absent, and by the presence of a single posterior siphonal opening, a fact that is in contrast with that reported for the type species of *Pseudokellya*, *Kellia cardiformis* Smith, 1885. The presence of a complete follicle surrounding each developing oocyte, persisting throughout vitellogenesis, a condition not currently known for other bivalves, is confirmed as a generic diagnostic character. The generic redefinition, based on shell morphology and anatomical features described for *Pseudokellya franki*, is given.

Additional Keywords: Cyamioidea, *Cyamiocardium*, *Kellia*, *Perrierina*, reproduction

INTRODUCTION

Pseudokellya was proposed by Pelseneer (1903) to realloeate *Kellia eardiformis* Smith, 1885, a speeies described from Kerguelen Islands. The diagnosis for the new genus was given in association with description of the anatomy of *Kellia eardiformis*. Speeies of *Pseudokellya* were originally distinguished from those of *Kellia* Turton, 1822, by having two, branehial and anal, siphonal openings and for being dioeeious. Pelseneer (1903) also reported a peeuliar reproductive trait for *Pseudokellya eardiformis:* the presence of a eomplete follieular epithelium surrounding eaeh developing oocyte, a condition not otherwise known for bivalves. Subsequently, four nominal speeies of *Pseudokellya* were deseribed: *Pseudokellya gradata* Thiele, 1912, from Gauss Station, *Pseudokellya stillwelli* Hedley, 1916, from Adelie Land and Davis Sea (a synonym of *P. cardiformis*, aeeording to Dell (1990)), *Pseudokellya georgiana* Dell, 1964, from South Georgia, and *Pseudokellya inexpeetata* Dell, 1964, from South Georgia and South Orkneys. The descriptions of these four species were based exclusively on shell eharacters; after that not a single study provided information on their anatomy. Consequently, to date, it is not possible to confirm if the anatomical characters reported by Pelseneer (1903) are diagnostie for *Pseudokellya eardiformis* or shared by other species of the genus.

In the present paper, a new speeies of *Pseudokellya* from South Shetland Islands is described; details on anatomy, reproductive traits and shell morphology provide additional information for a better definition of the genus.

MATERIALS AND METHODS

The specimens studied were originally deposited at the Zoologisches Museum (ZMB), Germany. They were collected during the 1982, 1985, and 1986 Soviet Antarctie Expeditions to King George Island, South Shetland Arehipelago. Voucher specimens are deposited at the ZMB, Museo de La Plata (MLP), and Museo Argentino de Ciencias Naturales (MACN), Argentina.

We studied the holotype of *P. gradata* (ZMB 63109), specimens of *P. cardiformis* (ZMB 63136), *P. inexpeetata* (ZMB 114683), and *P. georgiana* (MLP 12999) from type loealities, and speeimens of *P. gradata* from Zoologische Staatssammlung Münich (ZSM), Germany (ZSM 20012865: 63° 01.10′ S, 61° 09.10′ W; ZSM 20041320: 62° 00.09′ S, 60° 19.31′ W). Speeimens currently assigned to *Kellia suborbicularis* (Montagu, 1803) (MLP 11563); *Kellia magellaniea* Smith, 1881 (MLP 13000); and *Kellia* sp. (MLP 13001) from Argentine waters were also used for comparative purposes.

The anatomieal deseription of *Pseudokellya franki* was based on dissections under stereoscopic microscope; seven specimens were processed for histology, inclusion was performed either in Paraplast® or Historesin®; specimens were completely seetioned at 5 μm thick, using a Leica RM 2355 microtome.

Shell morphology was studied through scanning electron mieroscopy (SEM). Shell measurements were

obtained according to the following criteria: **L:** maximum antero-posterior distance; **H:** maximum dorsoventral distance perpendicular to L; **W:** maximum distance across valves. Mean value and standard deviation for the ratios **H/L** and **W/H** are given (n = 16 specimens). Hinge teeth nomenclature is indicated in figures 10 and 11.

SYSTEMATICS

Pseudokellya Pelseneer, 1903

Type Species: *Kellya cardiformis* Smith, 1885 (by monotypy)

Pseudokellya franki new species
(Figures 1–26)

Diagnosis: Shell subcircular, inflated, only sculptured with marked growth lines; periostracum forming lamellate commarginal folds. Posterior portion of the right cardinal tooth (**C3b**) well developed. A single posterior siphonal opening, the anal, present.

Description: Shell small, maximum observed **L** = 4.2 mm, shell outline subcircular, slightly longer than high (**H/L** = 0.94±0.04), inflated (**W/H** = 0.74±0.04) (Figures 2–5, 12). Anterior margin short and round, imperceptibly connected with dorsal margin, forming a wide curve with the anterior part of ventral margin (Figures 2–5). Ventral margin markedly curved. Posterior margin rounded, nearly vertical in larger specimens following the posteroventral curve (Figures 2–4). Posterior part of dorsal margin suberect or slightly curved. Beaks prosogyrous, inflated, globose at tip, strongly discernible above dorsal margin, slightly displaced anteriorly (Figure 2–5, 7). Prodissoconch ovate, about 400 µm in diameter, surface sculptured with minute granules (Figures 6, 13). Shell surface whitish, shiny, with very low and rounded commarginal growth lines, irregularly distributed (Figure 15). Periostracum translucent, forming low lamellate periostracal folds (Figure 14). Inner shell surface whitish, dull. Hinge plate narrow, somewhat enlarged anterior to beaks, just at the point of insertion of cardinal teeth (Figures 8–11). Hinge: left valve (Figures 8, 10): cardinal tooth 2 (**C2**) solid, triangular, cusp subcentral; cardinal tooth 4 (**C4**) relatively short, straight, and solid, with cusp displaced posteriorly; lateral posterior tooth (LII) long, narrow, and low, well separated from posterior margin. Right valve (Figures 9, 11): cardinal tooth (**C3**) hook-like, formed by large, solid anterior portion (**C3a**), bifid at base, and short and narrow posterior portion (**C3b**). In larger specimens, **C3a** and **C3b** form a nearly right angle (Figure 11), whereas in smaller ones the angle between **C3a** and **C3b** is more acute and **C3b** longer (Figure 9). Right inner posterior lateral tooth (**LI**) long, moderately solid, with centrally located cusp; outer posterior lateral tooth (**LIII**) merged with dorsal shell margin. Internal ligament set in a small, short, shallow resilifer posterior to cardinal teeth; external ligament short, posterior to beaks. Scars of anterior and posterior adductor muscles ovate, the anterior, slightly longer (Figure 7). Pallial line entire.

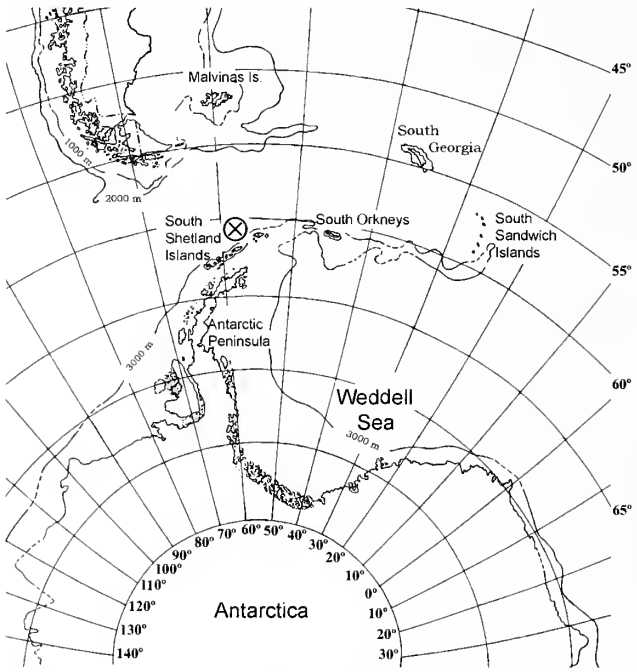
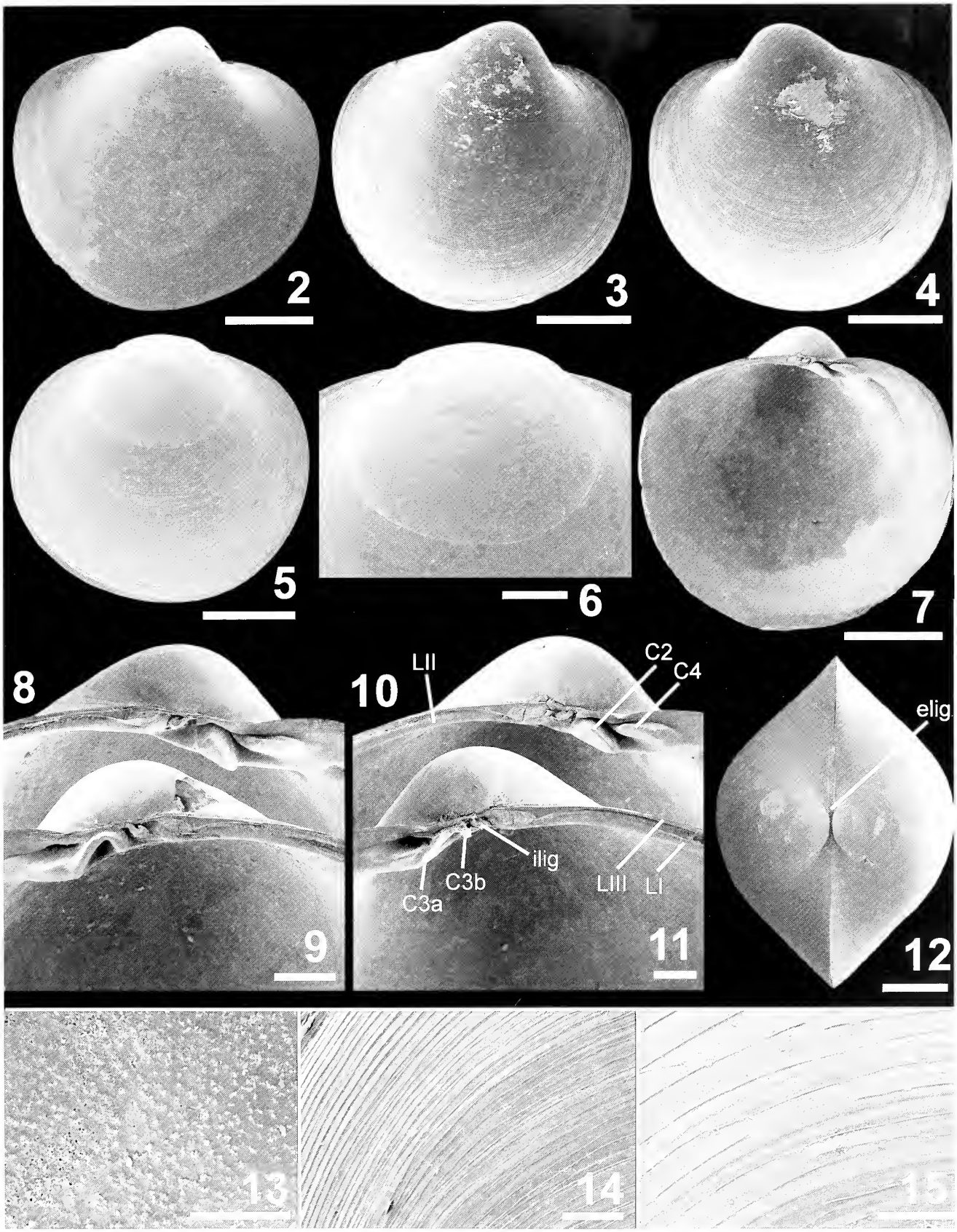


Figure 1. Location of the type locality of *Pseudokellya franki* new species.

Anatomy: Mantle margin largely unfused, forming a long pedal gape (Figure 20), fused at the posterior quarter, delimiting the anal opening; below this point, an enlarged portion of the middle mantle fold corresponds to the position of the absent branchial mantle opening (Figure 19). The anal opening and the enlarged portion of the mantle margin are flanked by a row of 12 to 16 micropapillate tentacles on each side, placed in an alternating pattern (Figures 18, 19). Anterior and posterior adductor muscles almost equal in size, the posterior one ovate in section, the anterior more elongated and narrower (Figure 16). Foot long, with a well differentiated heel (Figure 16); a small byssal gland, functional

Figures 2–15. *Pseudokellya franki* new species. Specimens from Maxwell Bay, King George Island, 100 m (station 30/49). **3, 4.** Holotype. **2, 5–15.** Other specimens. **2–5.** Outer view. **2, 3.** Right valve. **4.** Left valve. **5.** Juvenile. **6.** Prodissoeoneh. **7.** Inner view, left valve. **8–11.** Details of hinge plate. **8, 9.** Specimen 4.2 mm L. **10, 11.** Specimen 5.5 mm L. **8, 10.** Left valve. **9, 11.** Right valve. **12.** Dorsal view. **13.** Detail of prodissoconch sculpture. **14.** Periostraeal folds. **15.** Periostraeal folds and growth lines. Seale bars: 2–4, 7 = 1 mm; 5 = 250 µm; 6 = 100 µm; 8–11 = 200 µm; 12 = 500 µm; 13 = 25 µm; 14 = 100 µm; 15 = 50 µm. Abbreviations: **C2, C3a, C3b, C4** = cardinal teeth; **LI–LIII** = lateral teeth I–III; **ilig** = internal ligament; **elig** = external ligament.


2
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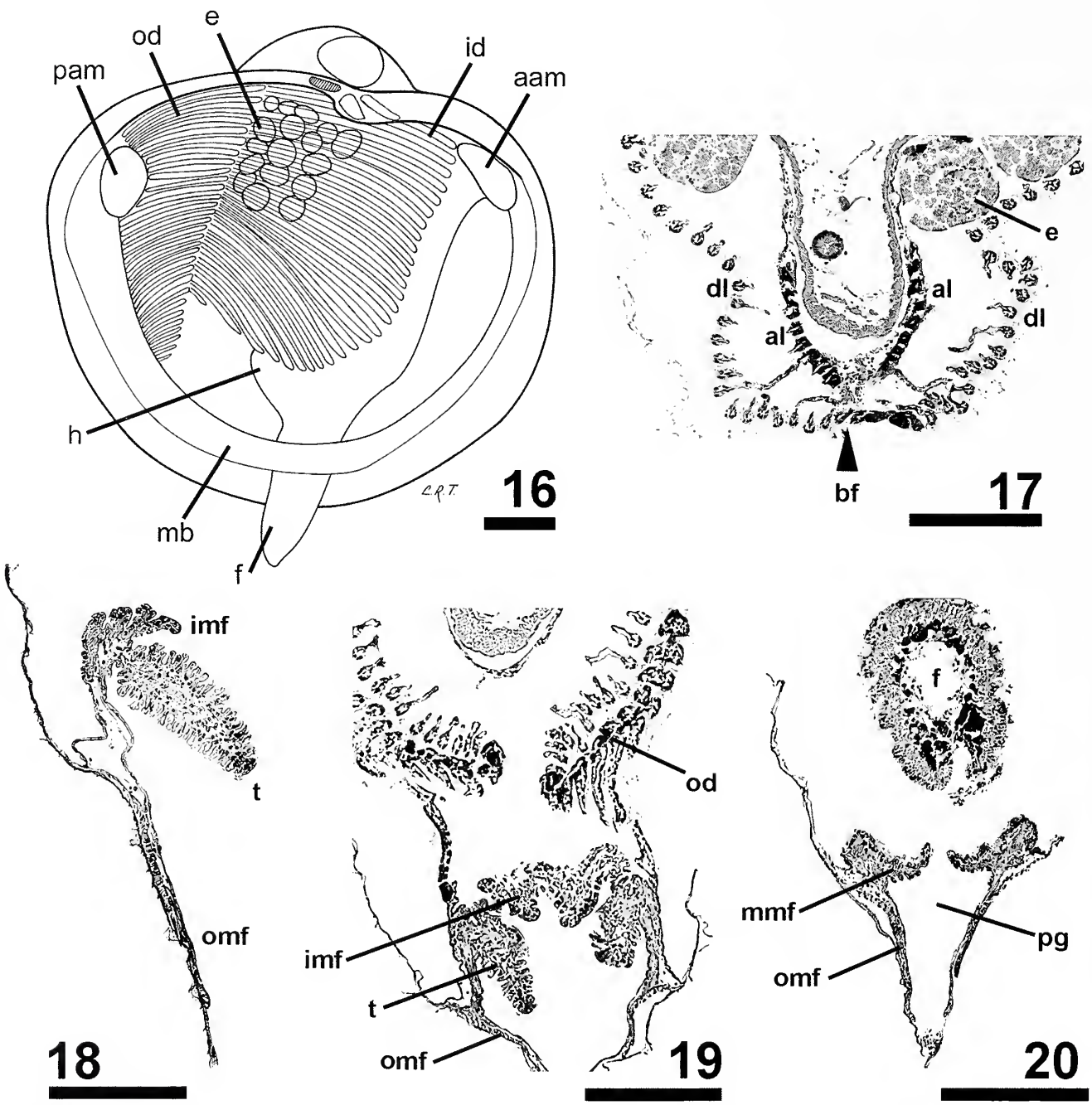
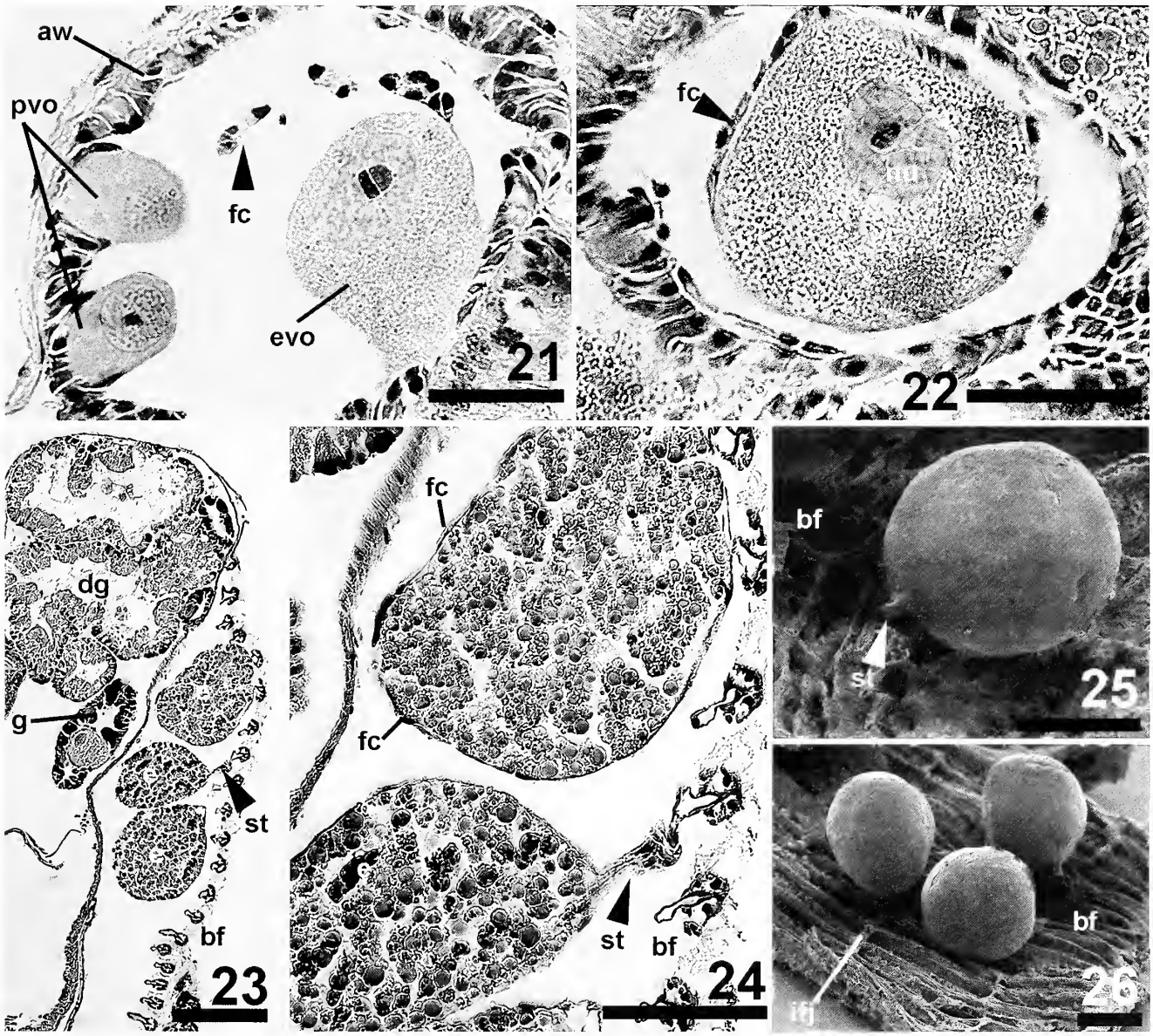


Figures 16–20. *Pseudokellya franki*: anatomy. **16.** Gross anatomy from the right side. **17–20.** Transverse seetions showing details of demibranch fusion and mantle border. **17.** Posterior fusion of the inner demibranchs. **18.** Detail of mantle folds and tentaele. **19.** Fusion of posterior portion of the mantle border. **20.** Anterior portion of the mantle border. Seale bars: 16 = 1 mm; 17, 19, 20 = 300 µm; 18 = 150 µm. Abbreviations: **aam** = anterior adduetor musele; **al** = ascending lamella of inner demibraneh; **bf** = branehial fusion; **dl** = deseending lamella of inner demibranch; **e** = embryo; **f** = foot; **id** = inner demibranch; **imf** = inner mantle fold; **h** = heel; **mb** = mantle border; **mmf** = middle mantle fold; **od** = outer demibraneh; **omf** = outer mantle fold; **pam** = posterior adductor musele; **t** = tentacle.

in adults, present; byssus comprising a single, long filament. Outer and inner demibranehs present, with well-developed ascending and descending lamellae (Figure 16). Height of outer demibranch representing one-third the height of inner one; posterior end of outer demibranch fused to the mantle; left and right inner demibranehs, also fused at posterior end, determining defining a suprabranehial ehamber eontinuous with the anal opening (Figure 17). Length of deseending lamella of outer demibraneh about a half of aseending one; filaments of ascending lamella of inner demibraneh deereasing in length toward the posterior end.



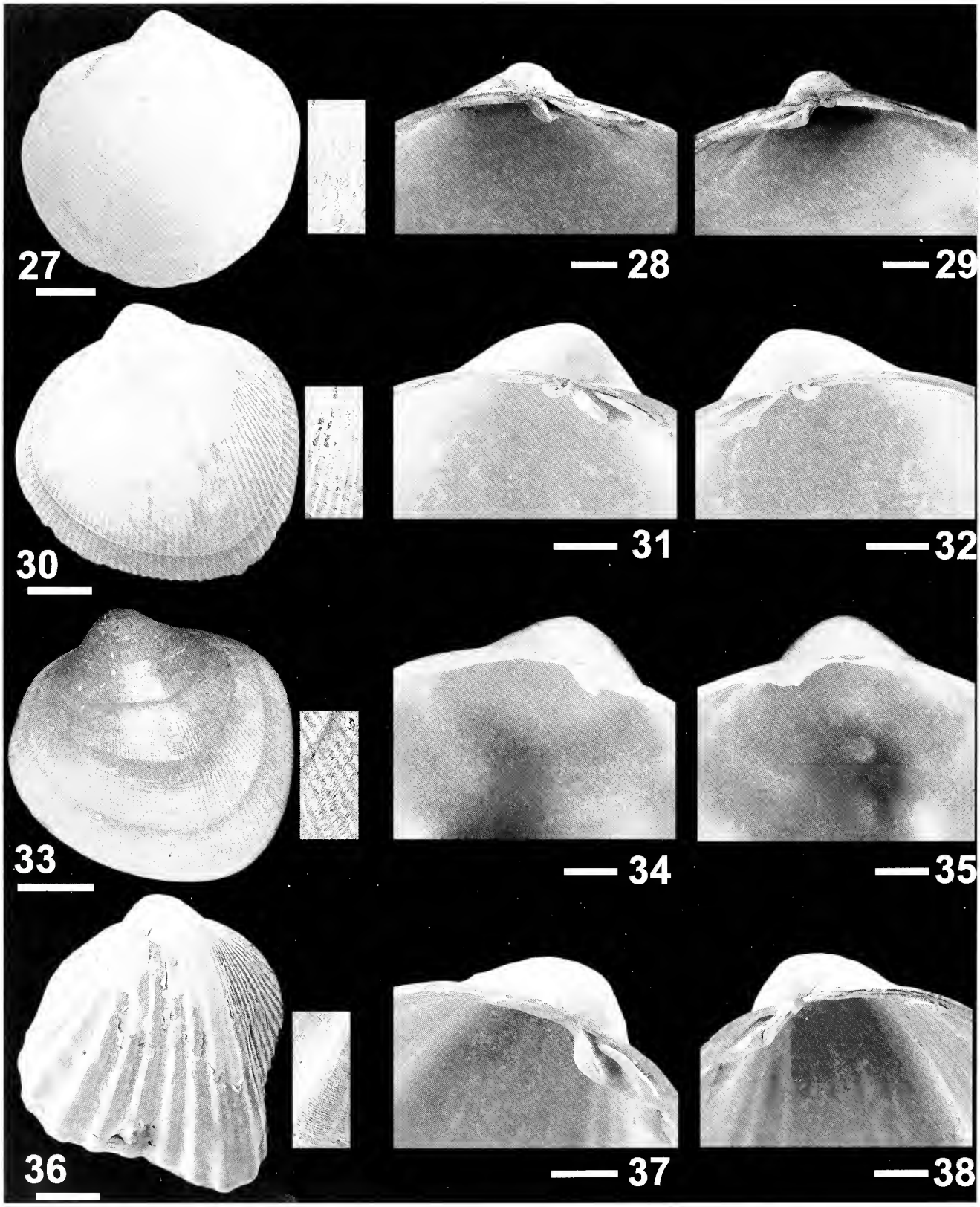
Figures 21–26. *Pseudokellya franki*: oocytes and embryos. **21–24.** Histological sections. **25, 26.** SEM photomicrographs. **21.** Pre-vitellogenic and early vitellogenic oocytes. **22.** Vitellogenic oocyte. **23–26.** Embryos attached to the inner demibranch. Scale bars: 21, 24–26 = 100 µm; 22 = 50 µm; 23 = 200 µm. Abbreviations: **aw** = acinar wall; **bf** = branchial filaments of the inner demibranch; **dg** = digestive gland; **e** = embryo; **evo** = early pre-vitellogenic oocyte; **fc** = follicle cell; **g** = gonad; **h** = heel; **ifj** = interfilamental junction; **nu** = nucleus; **pg** = pedal gap; **pvo** = pre-vitellogenic oocyte; **st** = stalk.

Reproductive Traits: *Pseudokellya franki* is dioecious, brooding its embryos within the inner demibranchs attached to the ascending filaments by short stalks (Figures 16, 23–26). The architecture of oogenesis shows a peculiar feature, consisting of the formation of a complete one-cell-thick follicle surrounding each developing oocyte, which persists to the end of vitellogenesis (Figures 21, 22, 24).

Type Locality: Maxwell Bay, 62°10–19′ S, 58°35–58′ W, King George Island, South Shetland Islands, 100 m (station 30/49).

Type Material: Holotype (ZMB 114680-a) and 10 paratypes from the type locality (five at ZMB 114680-b; three at MLP 12997; two at MACN-In 37535).

Other Material Examined: 36 specimens, Maxwell Bay, King George Island (station 30/43), 50 m (ZMB 114679); 84 specimens, Maxwell Bay, King George Island, 100 m (station 30/49) (ZMB 114680-c); 1 specimen, Maxwell Bay, King George Island, 10–15 m (station II/134) (ZMB 114682); 6 specimens, Maxwell Bay, King George Island, 40–50 m (station B 301) (ZMB 114681).



Figures 27–38. *Pseudokellya* species. **27–29.** *P. cardiformis* (ZMB 63136). **30–32.** *P. inexpectata* (ZMB 114683). **33–35.** *P. gradata*. **33.** Specimen from 62° 00.09′ S 60° 19.31′ W (ZSM 20041320). **34, 35.** Syntype (ZMB 63109). **36–38.** *P. georgiana* (MLP 12999). **27, 30, 33, 36.** Outer view left valve, and detail of shell sculpture at the right side. **28, 31, 34, 37.** Hinge plate left valve. **29, 32, 35, 38.** Hinge plate right valve. Scale bars: 27, 30, 33, 36 = 1 mm; 28, 29, 31, 32, 34, 35, 37, 38 = 500 µm.

Distribution: Only known from South Shetland Islands (Figure 1).

Etymology: The species is named after Frank Köhler, Australian Museum, Sydney, and associated with the Museum für Naturkunde, Berlin.

Remarks: In general shell outline, *Pseudokellya franki* is most similar to *Pseudokellya eardiformis* (Figures 27–29), from which it differs in having a relatively longer and more straight posterior part of dorsal margin and a ventral margin comparatively more strikingly curved. The shell outline of *P. georgiana* (Figures 36–38) differs from that of *Pseudokellya franki* in being markedly trapezoidal; *P. gradata* (Figures 33–35) and *P. inexpeetata* (Figures 30–32) have subtrapezoidal shell outlines.

The absence of radial sculpture on the outer shell surface is a distinctive character of *Pseudokellya franki* (Figures 2–5). Also distinctive in *P. franki* is the presence of widely separated and low lamellate commarginal periostracal folds (Figure 14); in other species of *Pseudokellya*, the periostracum shows densely packed and fine commarginal threads (Figures 27, 30, 33, 36). *Pseudokellya franki* shows a weak commarginal sculpture represented by low and rounded irregular ridges, which seems to originate through growth disruptions (Figure 15); in the remaining species this sculpture is less evident, being represented only by shallow growth lines (Figures 27, 30, 33, 36). In the case of *Pseudokellya gradata*, 3–4 sharp growth disruptions, described as "grades", appear (Figure 33).

The hinge of the largest specimens of *Pseudokellya franki* is similar to that of the other species of the genus, mainly differing in having a larger cardinal tooth 3b and a more solid cardinal tooth 2 with a triangular base. In the smaller specimens the cardinal 3 is arched, with **C3b** more developed.

Anatomically, *Pseudokellya franki* differs from *P. eardiformis* (the only other species in the genus for which anatomy is known) in having only one defined mantle opening, the anal, and a differentiated portion of middle mantle fold below the anal opening that seems to represent the inhalant branchial aperture (lacking only a ventral point of fusion delimiting the opening). Pelseneer (1903) reported two posterior siphonal openings in *P. eardiformis*. As it was described by Pelseneer (1903) for *P. eardiformis*, *P. franki* showed to be dioecious. Out of the seven specimens histologically studied, four were males and three females, with no signals of a possible consecutive sexuality detected.

TOWARD A BETTER DEFINITION OF *PSEUDOKELLYA*

The generic definition of *Pseudokellya* given by Pelseneer (1903) when describing *P. eardiformis* was based on three characters: the presence of two posterior (branchial and anal) siphonal openings, the dioecious condition, and a peculiar mode of oogenesis comprising the formation of a complete follicle surrounding each developing oocyte (a condition not known, at that times, for any other bivalve).

According to Thiele (1934: 858) the diagnostic characters of *Pseudokellya* are: "shell roundish or somewhat angular, uniformly bulging, with weak radial sculpture; umbo moderately elevated, situated in the center; hinge margin posterior to the ligament prolonged somewhat ridge-shaped; anterior hinge teeth of the left valve fairly long, diverging in an acute angle".

After the new information coming from the new species described here and the species described after the diagnosis by Thiele, an expanded redescription of the genus is needed.

Redescription of *Pseudokellya*: Shell small, shell outline subcircular to subtrapezoidal, ventral margin uniformly curved or more sharply curved at posterior half; beaks prosogyrous, subcentrally located. Prodissoconch sculptured with microscopic granules; teleoconch usually sculptured with a variable number of more or less marked radial cords, sometimes absent. Periostracum usually elevated in fine threads or low lamellate folds. Growth lines variably marked, sometimes looking like commarginal sculpture. Hinge plate narrow, enlarged anterior to beaks, just at the cardinal teeth insertion. Right valve with a hook-like cardinal tooth (**C3**), formed by an anterior part (**C3a**) varying from short and stout to long and slender, with a triangular base, bifid to a variable degree; and a smaller posterior portion (**C3b**), sometimes extremely reduced in size; a well-developed, elongated, narrow, and low inner posterior lateral tooth (**PI**), and an outer posterior lateral tooth (**PIII**) not well-separated from dorsal margin. Left valve: two cardinal teeth, the anterior (**C4**) running parallel to the anterior part of dorsal margin, and the posterior (**C2**), usually elongated and smaller, parallel or forming an acute angle with **C4**; a single and elongated posterior lateral tooth (**PII**), present. Resilifer small and shallow, located below beaks. Internal and external ligaments, present. Mantle with one or two posterior siphonal openings. Gills each composed of two demibranchs. Foot with a well-differentiated heel, having a small byssal gland. Animals dioecious, retaining the embryos attached by short stalks to the inner demibranch filaments; a complete follicle surrounds each developing oocyte throughout vitellogenesis.

Comparison with Other Genera: When describing *Pseudokellya*, Pelseneer (1903) focused in the presence of two siphonal (branchial and anal) openings and the dioecious condition, in oposition to *Kellia* which is a hermaphrodite and has only one posterior siphonal opening, the anal. It is to be noted that, in contrast to that described by Pelseneer (1903) *Pseudokellya franki* shows a single posterior mantle opening. Additional characters differentiating *Kellia* from *Pseudokellya* are found in the hinge morphology: the

former has a simple and triangular right cardinal tooth **C3** and two left cardinal teeth (**C2** and **C4**) arranged in a chevron pattern. Moreover, in *Kellia* both right and left posterior lateral teeth are consistently stronger than in *Pseudokellya*. In addition, *Kellia* only has the internal ligament. Lastly, the radial sculpture and periostracal folds present in some species of *Pseudokellya* are absent in *Kellia*.

The hinge of *Pseudokellya* closely resembles that of *Cyamiocardium* Soot-Ryen, 1951, and *Perrierina* Bernard, 1897, two genera also occurring in the Southern Ocean. However, in the last two an additional tooth behind the cardinal **C2** (referred to as cardinal tooth 4b) appears (Lamy, 1917; Zelaya, 2008; pers.obs.). Adult specimens of *Cyamiocardium* and *Perrierina* have an always well-developed **C3b**, which is reduced in size in larger specimens of *Pseudokellya*. *Perrierina* also has tubercles anterior and posterior to the beaks resembling a taxondont hinge, a character absent in members of *Pseudokellya* (see Zelaya, 2008).

The Geographic Distribution of *Pseudokellya*: Currently known species of *Pseudokellya* are restricted to Sub-Antarctic and Antarctic waters. According to Dell (1990), *P. cardiformis* and *P. gradata* are probably circumantarctic, extending to the Scotia Arc Islands and the former, reaching Malvinas and Kerguelen Islands. The remaining three species are restricted to the Scotia Arc islands: *P. inexpectata* known from South Georgia and South Orkneys Islands (Dell, 1964), *P. georgiana* from South Georgia (Dell, 1964), and *P. franki* from South Shetland Islands (present study).

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Phylogenetic position of the bivalve family Cyrenoididae—removal from (and further dismantling of) the superfamily Lucinoidea

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ABSTRACT

A molecular analysis using sequences from 18S and 28S rRNA genes of the brackish and freshwater bivalve *Cyrenoida floridana*, in conjunction with a wide range of other heterodont bivalves, demonstrated a close relationship with the families Corbiculidae and Glauconomidae and distant from the Lucinoidea, where the Cyrenoididae had been usually classified. Based on this result it is proposed that the Cyrenoididae be removed from the Lucinoidea, which, for living taxa, now includes only the family Lucinidae.

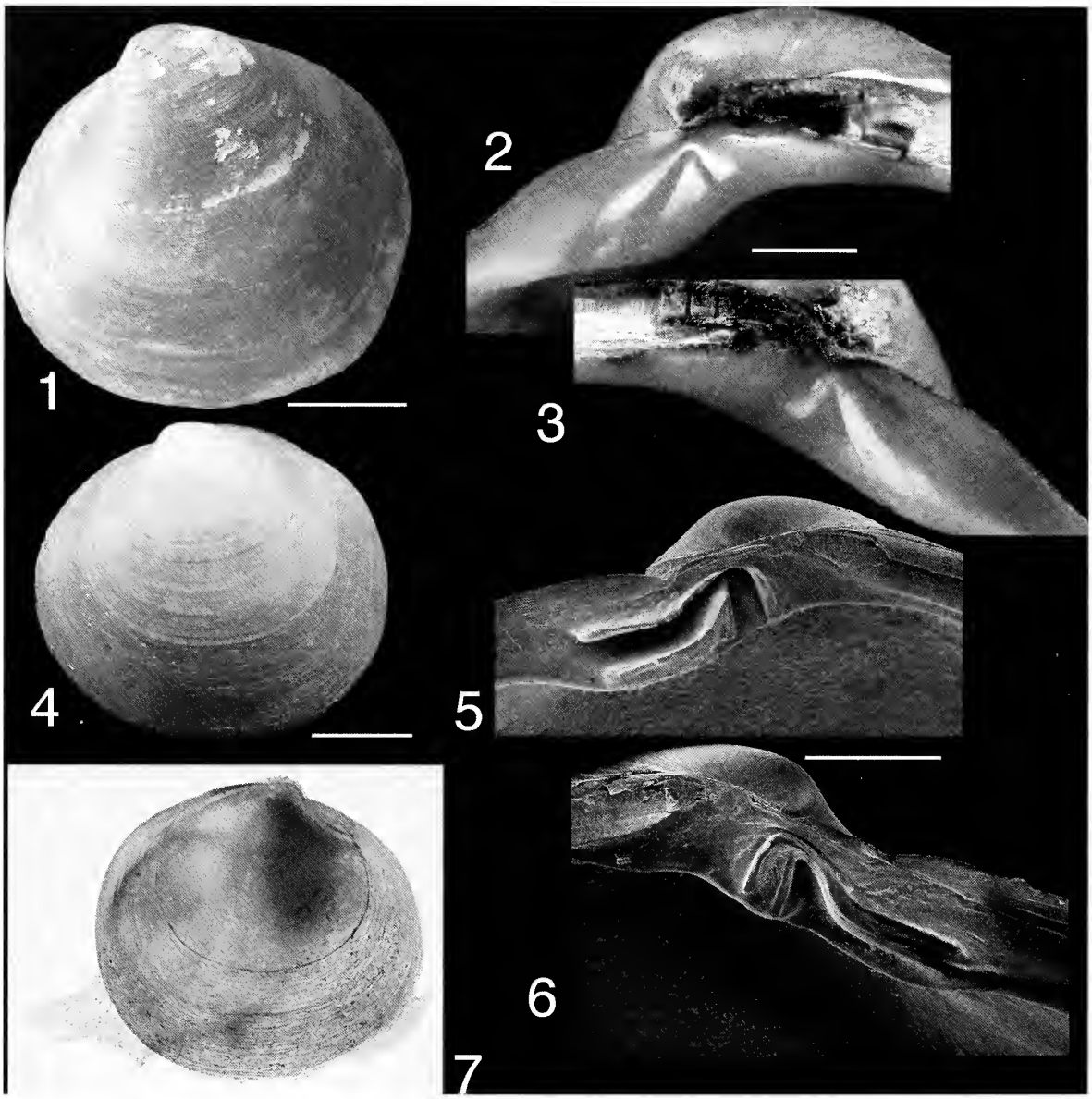
Additional Keywords: Bivalvia, Heterodonta, *Cyrenoida floridana*, 18S rRNA, 28S rRNA

INTRODUCTION

Until recently, most classifications of bivalve mollusks included within the superfamily Lucinoidea several component families (Lucinidae, Fimbriidae, Thyasiridae, Ungulinidae Cyrenoididae, and fossil Mactromyidae) (e.g. Dall, 1901; Chavan, 1969; Boss, 1982, Vaught, 1989; Amler, 1999). The Lucinidae and some Thyasiridae are notable for the chemosymbiosis with sulphide-oxidizing bacteria housed in the ctenidia (Southward, 1986; Taylor and Glover, 2006). Molecular analyses of the Lucinoidea, compared with a wide range of other heterodont bivalves, demonstrated that superfamily was not monophyletic, with the Thyasiridae and Ungulinidae not closely related to the Lucinidae (Williams, Taylor, and Glover, 2004; Taylor, Williams, and Glover, 2007; Taylor et al., 2007). The Ungulinidae group near families such as the Veneridae, Corbiculidae, and Mactridae, while the Thyasiridae form a basal clade within the Euheterodonta and are considered as a distinct superfamily—Thyasiroidea. *Fimbria fimbriata*, one of the two living species of Fimbriidae, nested together with Lucinidae species, with no support for separate familial status, and the nominal family was synonymized accordingly. Apart from the Lucinidae, the only other family with living species still classified within Lucinoidea is the Cyrenoididae, but lack of suitably preserved material has precluded inclusion in molecular analyses. From morphological evidence, Williams et al. (2004) and Taylor and Glover (2006) thought a relationship to the Lucinidae unlikely, *Cyrenoida* having medium to long fused siphons, well developed labial palps and ctenidia with two demibranchs. These statements led Bieler and Mikkelsen (2006) to place Cyrenoididae as *incertae sedis*.

The Cyrenoididae Adams and Adams, 1857 (= Cyrenellidae Gray, 1853) comprise a small group of around ten nominal species of little-studied bivalves inhabiting brackish to freshwaters, classified into a single genus, *Cyrenoida* (type species *C. dupontia* Joannis, 1835) (Figures 1–3), distributed in western Africa, eastern and western Americas and some islands of the Caribbean. The West African species inhabit brackish mangrove habitats (Pilsbry and Bequaert, 1927). In the eastern USA, *Cyrenoida floridana* Dall, 1901 (Figures 4–7) ranges from Delaware to the coast of the Gulf of Mexico, maybe as far west as Yucatan (Vokes and Vokes, 1983), where it inhabits fresh and brackish water habitats (Leathem, Kinner, and Maurer, 1976; Kat, 1982; Bishop and Hackney, 1987).

Dall (1895) was the first to place the Cyrenoididae within the Lucinoidea, stating (p. 545) "These are estuarine Lucinacea." Later (Dall, 1901: 817) stated ". . .shells of this group with a Lucinoid animal and *Diplodonta*-like shell, exhibit a hinge structure which is wholly distinct from any other of the Lucinacea." Many later classifications, including the influential Treatise of Invertebrate Paleontology (Chavan, 1969), followed Dall in placing the Cyrenoididae within the Lucinoidea (e.g. Vokes, 1980; Boss, 1982, Vaught, 1989, Skelton and Benton, 1993; Amler, 1999).



Figures 1–7. *Cyrenoida dupontia* (**1–3**) and *C. floridana* (**4–7**). **1.** *Cyrenoida dupontia* Joannis, left valve, Senegal (BMNH 20081055). Scale bar = 10 mm. **2–3.** Hinge teeth of *Cyrenoida dupontia*, right valve (**2**) and left valve (**3**). Scale bar = 2 mm. **4.** *Cyrenoida floridana* Dall, left valve, Blue Hole, Big Pine Key, Florida (BMNH 20081054). Scale bar = 2 mm. **5–6.** Hinge teeth (SEM images) of *Cyrenoida floridana*, right valve (**5**) and left valve (**6**) (BMNH 20081054). Scale bar = 500 µm. **7.** *Cyrenoida floridana*, living speeimen with short, fused siphons, Blue Hole, Big Pine Key, Florida. (Photo R. Bieler, September 2007).

Nevertheless, different opinions were expressed by other authors, Fiseher (1887: 1096), for example, placed Cyrenoididae (as Cyrenellidae) into a suborder Conehacea, near to *Corbicula* and Ungulinidae but apart from the Lucinoidea. While Thiele (1934) ineluded Cyrenoididae with other fresh and brackish water bivalves in the *stirps* Sphaeriacea but not positioned closely to Lucinoidea. The family was elevated to superfamily status by Olsson (1961: 227) but placed near to Lucinoidea, a decision also followed by Keen (1971). The superfamily Cyrenoidoidea was also recognized by Nevesskaya et al. (1971) and plaeed along with Lueinoidea in the order Astartida. Alternatively, and rather bizarrely, Starobogatov (1992) plaeed Cyrenoidoidea within the infraorder Eryeinoinei along with Cyamioidea, Galeommatoidea and Leptonoidea, all eontained within the order Lueiniformes.

Clearly, there exists mueh uneertainty concerning the phylogenetie position of Cyrenoididae amongst the heterodont bivalves but this has never been tested by either morphological or molecular analyses. In 2007, we obtained samples of *Cyrenoida floridana* suitable for molecular analysis and in this paper we present 18S and

28S rRNA sequenees for the species that enable us to establish the phylogenetic position of the family in relation to a wide range of heterodont bivalve taxa previously analysed (Taylor et al., 2007) and specifically address the question of whether the Lucinidae and Cyrenoididae form a monophyletic group.

MATERIALS AND METHODS

The sample of *Cyrenoida floridana,* preserved in 100% ethanol (BMNH 20081053), was eollected (18 September 2007) from Blue Hole (24°42.4′ N, 81°22.8′ W) a freshwater pond on Big Pine Key, Monroe County, Florida Keys, Florida, USA, from shoreline mud up to 0.5 m depth among roots of marginal reeds. Other material from the same site is lodged at the Field Museum of Natural History (FMNH 314434; 317667).

For the moleeular analysis, methods of DNA extraetion, amplifieation and sequencing followed by sequence analysis and phylogenetic reconstruction are as described in Taylor et al. (2007). Sequenees for *Cyrenoida floridana* were analysed together with the data set of heterodonts listed in Taylor et al. (2007, Table 1), with the addition of new 18S and 28S sequenees for *Mya arenaria* Linnaeus, 1758 (family Myidae) from Gydnia, Poland. The new sequences for *Cyrenoida floridana* and *Mya arenaria* are lodged in GenBank (Aeeession numbers: *C. floridana* FM999789, FM999790; *M. arenaria* FM999791, 779792). Voucher speeimens of both species are housed in the Department of Zoology, The Natural History Museum, London.

Phylogenies were constructed using Bayesian methods (MrBayes v3.1.2, Huelsenbeek and Ronquist, 2001) using a GTR+G+I model. The analysis for each data set was run for 3,500,000 generations, with a sample frequeney of 100. Eaeh analysis was run twiee. The first 15,000 trees from each run were disearded so that the final eonsensus tree was based on the eombination of aeeepted trees from eaeh run (a total of 40,000 trees). Support for nodes was determined using posterior probabilities (PP, caleulated by MrBayes).

RESULTS

The eombined tree based on concatenated sequences from 18S rRNA and 28S rRNA genes is shown in Figure 8. The individual trees based on single genes are very similar in topology to those published previously (Taylor et al., 2007). In all analyses *Cyrenoida floridana* nests in a highly supported clade with *Corbicula fluminea* (Corbiculidae) and *Glauconome virens* (Glauconomidae). This clade forms part of a major group of heterodonts named Neoheterodontei by Taylor et al. (2007). *Cyrenoida* is widely separated from both Thyasiridae and Lueinidae that appear in the more basal parts of the tree. The Ungulinidae, although also a member of the Neoheterodontei, form a separate elade distinet from *Cyrenoida.*

DISCUSSION

It would have been desirable to have included the type speeies of *Cyrenoida,* namely, *Cyrenoida dupontia* Joannis, 1835, from West Africa, in the molecular analysis but no suitably preserved material was available. Although a much smaller species, *C. floridana* is similar to *C. dupontia* in shell characters, notably the unusual hinge dentition, and we feel confident that they are members of the same gronp. *Cyrenoida dupontia* has three cardinal teeth in the right valve, the anterior of these is thin and elongate and the central tooth larger and slightly bifid (Figures 2–3) while the left valve has two cardinals, the posterior tooth smaller and bifid and the anterior tooth elongate. Lateral teeth are absent. The dentition of *C. floridana* is very similar (Figures 4–6) with three cardinal teeth in the right valve, the central being larger and two eardinal teeth in the left valve with the posterior tooth bifid and the anterior tooth elongate. We have also examined the gross anatomy of *Cyrenoida rosea* (d'Ailly, 1896) from Nigeria (National Museum of Wales specimen NMW.Z.2003.029.02041) and this has ctenidia with two demibranchs, with the inner demibranch larger, paired triangular labial palps, and fused medium-length posterior siphons. *Cyrenoida floridana* is similar, with small outer demibranchs, triangular labial palps and short fused posterior siphons, the inhalant with papillae (Figure 7). Despite the presence of siphons, there is no pallial sinus in any *Cyrenoida* speeies.

The main conclusion of this study, based on our results for *Cyrenoida floridana,* is that the Cyrenoididae should be removed from the Lucinoidea and classified close to, or possibly within, the Corbiculoidea. The status of *Cyrenoida* in relation to Corbiculidae and Glauconomidae needs further analysis with a larger dataset of corbiculid species. For the present the family can be classified within a separate superfamily Cyrenoidoidea as proposed by Olsson (1961). Molecular evidence for a highly supported relationship between Corbiculidae and Glauconomidae was reported by Taylor et al. (2007) although the elongate shells with deep pallial sinus and long siphons of *Glauconome* are less similar morphologically to Cyrenoididae and Corbiculidae. Species of Cyrenoididae and Corbiculidae occur in both brackish and freshwater habitats while Glauconomidae live intertidally among mangroves in environments of fluctuating salinity.

For living taxa, we consider that the superfamily Lucinoidea should now include only the family Lucinidae, with the families Thyasiridae, Ungulinidae and Cyrenoididae exeluded. The position of the entirely fossil families Maetromyidae, Ilionidae, and Paraeyclidae is unresolved although the latter two embrace species with lucinid characters.

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It is pleasure to thank Rüdiger Bieler and Petra Sierwald for generous hospitality and help in collection of

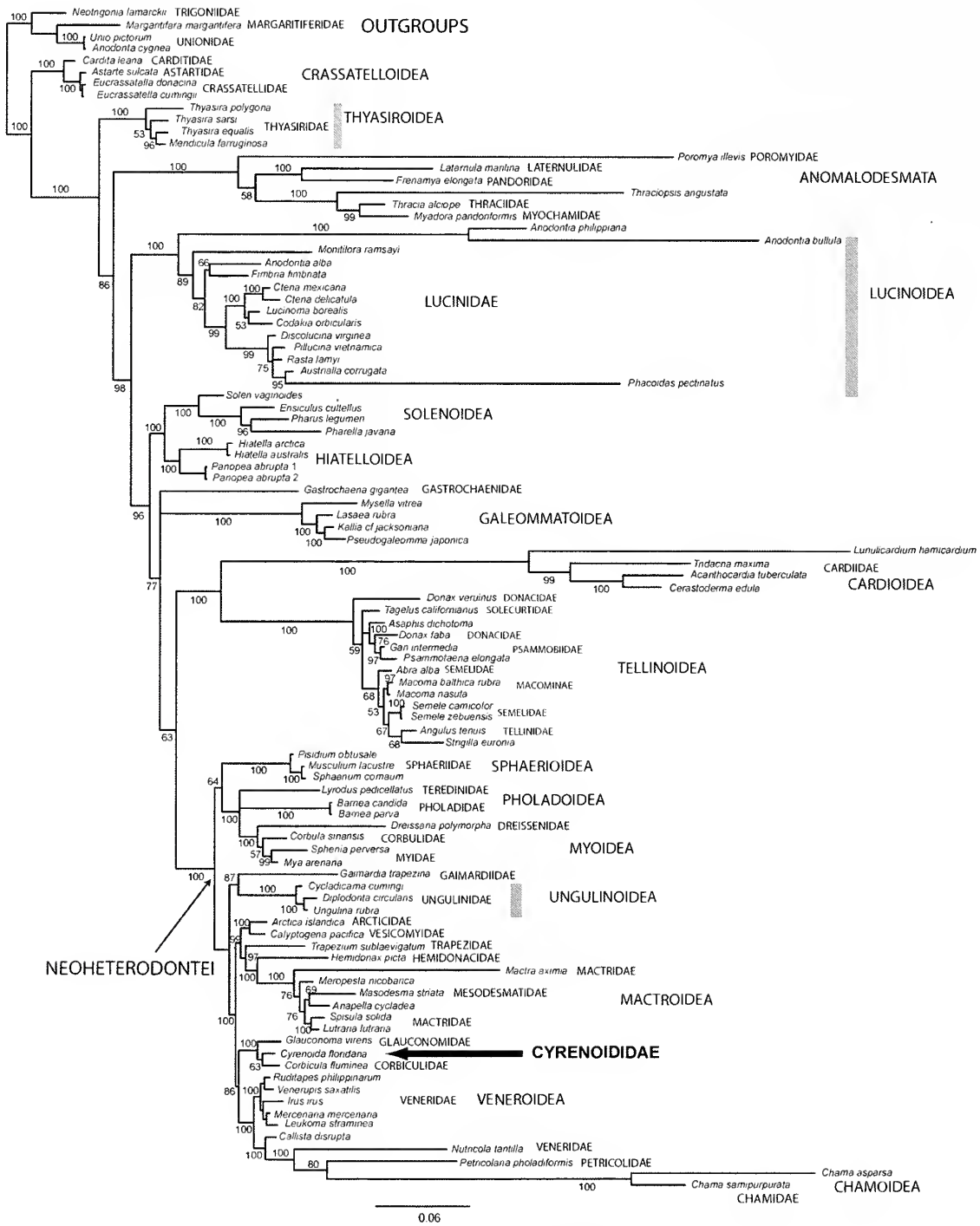

Figure 8. Molecular phylogeny of heterodont bivalves including *Cyrenoida* produced by Bayesian analysis for concatenated sequences from 18S and 28S rRNA genes. The tree was drawn using members of the palaeoheterodonts Trigoniidae, Unionidae, and Margaritiferidae as outgroups. Support values are posterior probabilities. Nodes with <50% support have been collapsed. Positions of Lucinoidea, Thyasiroidea, and Ungulinoidea marked by grey bars. Details of taxa in Taylor et al. (2007).

C. floridana. We are grateful to Pat Dyal for assistance with molecular analysis and to Graham Oliver (National Museum of Wales) for making a specimen of *Cyrenoida rosea* available. We thank Professor Phil Rainbow and Department of Zoology, NHM for continuing support. Research on this exemplar species in the Bivalve Tree-of-Life project (www.bivatol.org <http://www.bivatol.org/>) was supported by the U.S. National Science Foundation AToL program (DEB-0732854/0732903/0732860). The specimens were collected under U.S. Fish and Wildlife

Service Special Use Permit 41580-2007-11 (to Rüdiger Bieler) for research on native molluscan species in the National Key Deer Refuge.

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Establishment and persistence of the copse snail, *Arianta arbustorum* (Linnaeus, 1758) (Gastropoda: Helicidae) in Canada

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ABSTRACT

Although recorded from Newfoundland in 1885, by the late 1930s the copse snail, *Arianta arbustorum* (Linnaeus, 1758), was believed to no longer be extant in North America. We investigated sites in Newfoundland, New Brunswick, and Ontario, Canada and found that *A. arbustorum* is well established in these provinces; extant populations have persisted in Newfoundland for at least the past 30 years and in Ontario for more than 50 years. Where present in Canada, the species may sometimes be abundant, although populations are quite local, sometimes occupying less than 1 ha. Canadian Food Inspection Agency records show *A. arbustorum* to have been intercepted regularly (0–3 interceptions/year) since record-keeping started in 1963. Interceptions have occurred in 7 provinces spanning the country from Nova Scotia to British Columbia. Nursery stock originating in the Netherlands appears to be the main vector, but preliminary molecular data from Newfoundland populations suggests multiple European points of origin.

Additional Keywords: Introduced species, invasive species, Newfoundland, New Brunswick, Ontario

INTRODUCTION

The copse snail, *Arianta arbustorum* (Figure 1), occurs commonly across northwestern and central Europe (Kerney and Cameron, 1979). Despite this widespread European distribution, and the significant number of European molluscan taxa now found in North America (Robinson, 1999), *A. arbustorum* has apparently never become established in the United States and has only rarely become established in Canada. Dundee (1974), in a list of introduced mollusks of eastern North America, noted interceptions of *A. arbustorum* by the US Department of Agriculture at ports in six eastern states, but neither Mead (1971) nor Dundee (1974) reported any established populations. Until recently, the only published North American record of *A. arbustorum* is an 1885 observation from St. John's Newfoundland, reported by Whiteaves (1904). Grimm (1996) mentioned the occurrence of one colony in a ravine in Toronto, Ontario, but provided no details. Here we document the occurrence of *A. arbustorum* in Newfoundland, New Brunswick and Ontario and confirm the persistence or reintroduction of this species in Ontario and Newfoundland. We also review records of non-native plant pests intercepted by federal authorities from across Canada and show that *A. arbustorum* has been regularly imported into the country for more than 40 years.

MATERIALS AND METHODS

Following discovery of *A. arbustorum* in western Newfoundland by RGN in 1970 and its subsequent discovery in New Brunswick in 2004, we accumulated data in each region in order to delimit local distribution. We also examined specimens deposited by the late F. W. Grimm in the Canadian Museum of Nature and consulted his unpublished field notes at the Bishops Mills Natural History Centre, for reference to Ontario occurrences. These notes plus additional occurrence information from entomologist D. Monty Wood, one of Grimm's correspondents, led DFM and FWS to make confirmatory searches of ravine sites in Toronto. In addition, Canadian Food Inspection Agency records, maintained since 1963, were examined and all interceptions of *A. arbustorum* noted, along with country of origin, number of snails intercepted, and plant host. Voucher material of *A. arbustorum* collected during this study is deposited in the collections of the Bishops Mills Natural History Centre (EOBM), the New Brunswick Museum (NBM), and the Provincial Museum of Newfoundland and Labrador (NFM).

Figure 1. Live *Arianta arbustorum*, central Saint John, New Brunswick, May 2007. Scale bar = 1 cm. Photo M. Sollows, 2007.

RESULTS

Ontario: Collections data (Canadian Museum of Nature 059910 and Field Museum of Natural History 267829) provide more details on the Ontario population reported by Grimm (1996); material was collected from the Lawrence Park School complex on the north slope of Chatsworth Ravine, Toronto, 14–15 October 1970 by F.W. Grimm and J. Cavanaugh (43.720° N, 79.406° W; Figure 2a). Grimm's field notes also indicate material was collected by D.M. Wood from Rosedale Ravine, about 6 km away, around 1950. Unfortunately, vouchers from this site no longer exist. However, Wood recently stated his material was collected opposite Parliament Street on the north side of the ravine at 7 Dale Avenue (43.673° N, 79.372° W, pers. comm. to DFM). Searches of the Chatsworth Ravine by FWS (May 2006) and DFM (September 2007) revealed that the population is still extant, at least on the basis of numerous fresh-dead shells; however, *A. arbustorum* (EOBM 1667; NBM 367; Figure 3) were uncommon relative to the co-occurring *Cepaea nemoralis* (Linnaeus, 1758). Dead shells were restricted to an area of ~1.5 h within the Chatsworth Ravine. Searches of the Rosedale Ravine by DFM and FWS in August and September 2007 revealed no *A. arbustorum*, although urban development and gated and fenced properties precluded our access to some areas.

New Brunswick: In 2004, DFM and MCS found a well established population of *A. arbustorum* in central Saint John (Site 2, Figure 2b; 45.270° N, 66.078° W; NBM 143, 8602). Collections were subsequently made elsewhere in the city (Greenhead, 45.267° N, 66.133° W; NBM 136; Figure 4; west Saint John, 45.249° N, 66.062° W; NBM 315). Locally, the species is abundant. Collection sites are 0.3–5.25 km apart and collectively encompass an area of ~20 h straddling the St. John River (Figure 2b).

Newfoundland: In 1970, RGN collected *A. arbustorum* from an urban garden close to the trans-island railway corridor on Chapel Hill (road), Deer Lake, in western Newfoundland (Figure 2a; 49.16° N, 57.43° W; NFM MO-1971, 1972; [all lat/longs reported here are consistent with NAD83]) and from close to the Deer Lake Airport (49.1917° N, 57.4083° W; NFM MO-1970), but JEM was unable to relocate either of these populations in 2006. In 1976, RGN collected *A. arbustorum* at Petty Harbour-Maddox Cove (northern site) near St. John's (Figure 2c; 47.4853° N 52.7049° W; NFM MO-1973). In 1984, RGN and JEM again collected *A. arbustorum* from this locality (NFM MO-389). Subsequent observations and collections of the species in the general St. John's area by JEM and RGN between 1986 and 2007 include: Three Island Pond between Torbay and Bauline (47.6767° N, 52.7778° W), just east of Lundrigan's Marsh (47.6031° N, 52.6813° W), Kent's Pond (47.5864° N, 52.7242° W), a hydro pole-line near Oxen Pond Road (47.5825° N, 52.7535° W), Masonic Terrace (47.5656° N, 52.7072° W), Syme's Bridge (47.5433° N, 52.7244° W; Figure 5), Bowring Park (northern site) (47.5279° N, 52.7447° W), Bowring Park (southern site) (47.5222° N, 52.7547° W; NFM MO-684, 1370, 1391) and Petty Harbour-Maddox Cove (southern site)(47.4682° N, 52.7067° W).

The total Newfoundland population of *A.arbustrorum* presently occupies ten small localities (collectively covering ~1 ha; Figure 2c) within a narrow area of about 23.5 × 6 km radiating north-south from the original 1885 discovery site at the entrance to St. John's Harbour.

Canada Food Inspection Agency Interceptions: Table 1 summarizes records of *A. arbustorum* intercepted by the Canadian Food Inspection Agency at Canadian inspection stations since 1963. The species has been reported on 26 occasions (range of 0–3 interceptions/year) at stations in Nova Scotia, New Brunswick, Québec, Ontario, Saskatchewan, Alberta, and British Columbia, usually in association with a variety of garden plants imported from the Netherlands (73%), or, less commonly, other European countries (23%).

DISCUSSION

Whiteaves (1904) reported *A. arbustorum* from "grassy slopes facing the sea near the narrows of St. Johns Harbour, Newfoundland" in mid-July 1885. However, Brooks (1936) and Brooks and Brooks (1940) noted that their searches of the area around St. John's in 1934 did not reveal the species. Pilsbry (1939) suggested that the Newfoundland population might no longer persist and deemed the species a "rather doubtful member of the American fauna". The Petty Harbour-Maddox Cove population was very small when discovered in 1976, and does not appear to have spread much since. The Petty Harbour-Maddox Cove localities are physically separated from the eight remaining sites by the steeply rising 200+ m north-south trending Southside Hills. Likewise, the Bowring Park (southern site) population was also very small when it was discovered in 1986, restricted to the grounds of an old estate. However, it appears to have

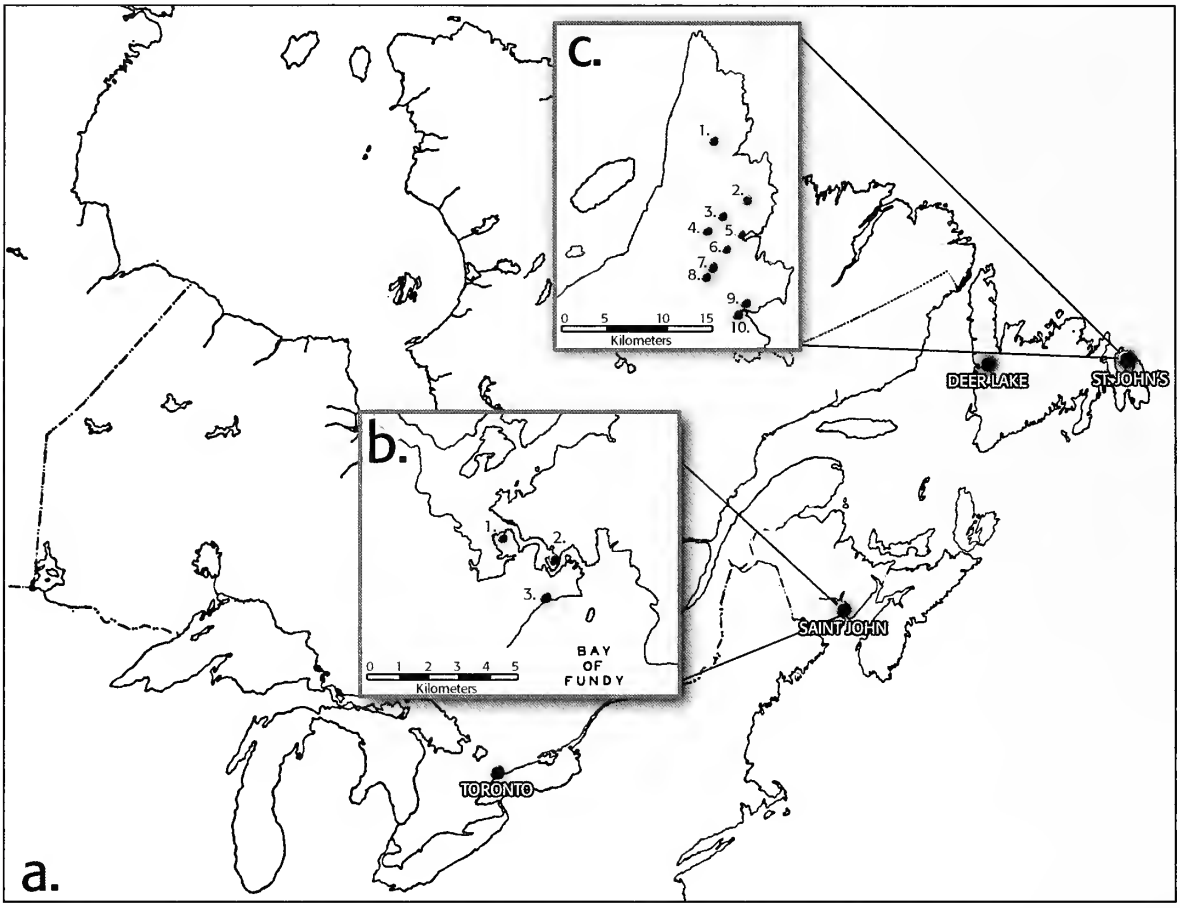


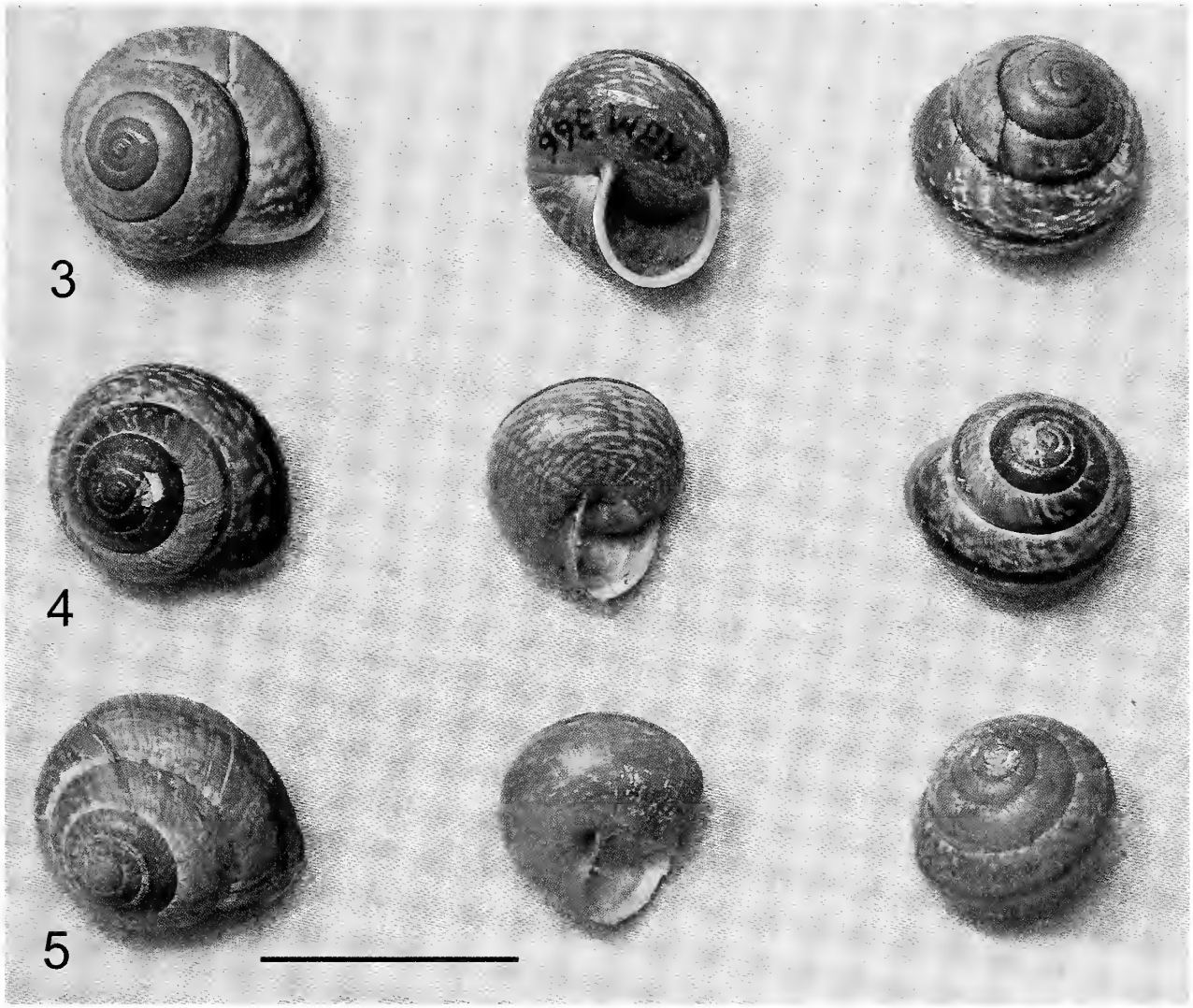
Figure 2. a. Eastern North America showing sites (•) in Ontario, New Brunswick and Newfoundland where *Arianta arbustorum* has been reported. b. Saint John, New Brunswick with sites of occurrence for *A. arbustorum*; 1. Greenhead; 2. Saint John central; 3. Saint John west. c. St. John's, Newfoundland and environs with sites for *A. arbustorum*; 1. Three Island Pond; 2. Lundrigan's Marsh; 3. Kent's Pond; 4. Oxen Pond; 5. Masonic Terrace; 6. Symes's Bridge; 7. Bowring Park north; 8. Bowring Park south; 9. Petty Harbour-Maddox Cove north; 10. Petty Harbour-Maddox Cove south.

spread significantly during recent years and is probably the source of the eight occurrences now known in the main St. John's area. Preliminary molecular data suggest that the present-day Newfoundland populations are derived from at least two separate introductions from Europe; the Petty Harbour-Maddox Cove populations being genetically distinct from the greater St. John's area populations (A. Grindon, Nottingham University, pers. comm. to DFM). The Deer Lake records may represent ephemeral populations derived from snails transported from St. John's in rail cargo, since both localities are located along the former trans-island railway corridor.

Although the area occupied by *A. arbustorum* in New Brunswick suggests a long-standing population, it is not possible to estimate a likely date of introduction for *A. arbustorum* to Saint John. Matthew and Stead (1903) made no mention of the species in their list of land and freshwater mollusks collected in and near Saint John about 1890–1900. Unfortunately, the mollusk survey of Coleman (1966) conducted in Saint John is incomplete, even for the marine and freshwater species sampled.

An undated collection record in the Field Museum of Natural History (FMNH 38439) reports a single dry shell from "Selkirk, New Brunswick". A search of recent and historical gazetteers reveals no such location in that province. The specimen and original label appear to be missing. The specimen was originally in the collection of G.K. Gude, a malacologist resident in the United Kingdom, who was active in the early 20th Century. Gude produced very small labels and it seems quite likely that he would have abbreviated his label data (J. Gerber, pers. comm. to DFM). We suggest that this record as reported is the result of an error in transcription. While it may refer to Selkirk, Manitoba (MB), rather than New Brunswick (NB), the record may not even be North American. Gude undoubtedly exchanged widely; however his research interests focused on regions outside the North American continent, and there is no material from the Gude Collection now in the Field Museum labeled as being from Manitoba.

Considering that *A. arbustorum* is widely distributed and common in Europe, and appears to be imported not



Figures 3–5. Representative specimens of *Arianta arbustorum* from **3.** Ontario (NBM 366), **4.** New Brunswick (NBM 355), and **5.** Newfoundland (NBM 8355). Scale bar = 2 cm.

infrequently into Canada, it is surprising that the species has not been recorded as more widely established in temperate regions of North America. Robinson (1999) listed the species as an uncommonly imported invasive, accounting for <0.1 % of more than 4,900 US interceptions over about a 6-year period. Although this percentage is small, it still accounts for a sizable number of animals. In Canada, <5% of tropical plants from the United States are examined but 100 % of off-continent nursery stock is inspected (D. Parker, pers. comm. to DFM). One interception at Edmonton, Alberta, in 1999–2000 originated at Goulds, South Florida. As there are no vouchers for this interception the identification cannot be confirmed. However, given the species restriction to north-temperate latitudes in Europe, establishment of *A. arbustorum* in Florida seems unlikely.

In summary, *A. arbustorum* may have persisted for more than a century on Newfoundland or may have been repeatedly introduced; preliminary evidence indicates multiple introductions. It has also been present in New Brunswick and Ontario for some time. Further investigation may reveal that this European alien is more widely distributed at temperate latitudes in North America than was previously thought.

ACKNOWLEDGMENTS

We thank D. Parker, Head, Identification and Regulatory Entomology, Canada Food Inspection Agency, Ottawa, for making records of intercepted plant pests available to us. D.M. Wood for sharing his knowledge of the Rosedale Ravine population with us. N. Djan-Chékar, Provincial Museum of Newfoundland and Labrador, J.-M. Gagnon, Canadian Museum of Nature, Ottawa, and J. Gerber, Field Museum of Natural History, Chicago, for access to collections data and responded

Table 1. Canadian records of interceptions of *Arianta arbustorum* 1963–2005.

Year	Location	#	Host Origin
1964–65 Belgium	Ontario	1	*Quercus*
1965–66 Netherlands	Quebec	1	*Lonicera*
1966–67 Belgium	Saskatchewan	1	*Berberis*
1967–68 Europe; Monaco	Quebec	2	Herbaceous plants
1968–69 Belgium	Quebec	2	*Acer;* packing with plants
1969–70 Netherlands	British Columbia	I	*Aesculus*
1970–71 Netherlands	Quebec	1	Packing with plants
1971–72 Netherlands	British Columbia	1	Packing with plants
1972–73 Netherlands	Nova Scotia	1	*Pinus*
1976–77 Netherlands	Saskatchewan	1	*Sambucus*
1979–80 Netherlands	British Columbia	1	*Spiraea*
1980–81 Netherlands	Quebec; British Columbia	3	*Caragana*; *Malus;Rosa*
1982–83 Netherlands	Quebec	1	Packing with plants
1986–87 Netherlands	Québec; Saskatchewan; Alberta	3	*Caragana; Rosa;Viburnum*
1989–90 Netherlands	Quebec; British Columbia	3	Plants; *Sambucus*; *Sphagnum*
1998–99 Netherlands	Quebec	1	*Caragana*
1999–2000 Florida	Alberta	1	*Ficus*
2004–05 Netherlands	New Brunswick	1	*Tilia*

to our enquiries. A. Grindon, Nottingham University for preliminary molecular data. R. Forsyth drew our attention to the Selkirk record in the Field Museum of Natural History.

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Research Note

Rediscovery of a Caribbean living fossil: *Pholadomya candida* G.B. Sowerby I, 1823 (Bivalvia: Anomalodesmata: Pholadomyoidea)

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Pholadomya candida G.B. Sowerby I, 1823, is an anomalodesmatan bivalve belonging to the ancient family Pholadomyidae, a group of burrowing bivalves with a wide palaeobiogeographic distribution from the Carboniferous to the Recent (Cox, 1969). This group is characterized by having posteriorly elongated shells with strong radial ribbing, a siphonal gape, a hinge with no functional teeth, and an external opisthodetic ligament (Runnegar, 1974).

Although several recent species from different regions of the world have been described under *Pholadomya*, the type species, *P. candida*, is the only Recent species resembling a great number of fossil forms in size, shape, and life habits (Cox, 1969; Waterhouse, 1969; Lazo, 2007). Accordingly, all other extant members of the Pholadomyoidea are more correctly placed in the genera *Parilimya* Melvill and Standen, 1899 or *Panacca* Dall, 1905 (Cox, 1969; Runnegar, 1972; Zinsmeister, 1978; Morton, 1980; Lazo, 2007). It seems then that *P. candida* is the only remaining species of a genus that flourished for more than 200 million years from the Late Triassic to the Recent, and this is why Runnegar (1972, 1979) and Morton (1980) have referred to it as a "living fossil."

Records of *Pholadomya candida* are extremely scarce, mostly from the West Indies. Since living specimens had not been found since the latter part of the 19th Century, the species was considered as possibly extinct (Runnegar, 1972; Morton, 1980). However, discoveries of fresh-looking shells from Venezuela (Gibson-Smith and Gibson-Smith, 1980) and Colombia (Díaz and Borrero, 1995) provided evidence that it still may be living, at least in the southern Caribbean.

Pholadamya candida had been collected alive only twice, at least in the sense of being available for scientific studies (Morton, 1980); both specimens were found before 1842 in the same area, the Virgin Islands. One of them was dissected by R. Owen in 1839, but some of his illustrations were lost and his manuscript never published (Runnegar, 1972). The second specimen was dissected and the functional anatomy described by Morton (1980).

On November 2004, while diving at Bahía Concha, a sheltered bay near Santa Marta, on the Caribbean coast of Colombia (11°17′56″ N, 74°08′52″ W), a pair of openings on the sandy bottom at a depth of about of 4 m caught the attention of one of us (DCT). Excavating deeply around the holes, she exposed a large clam (about 20 cm long) bearing white, pearly valves that she hadn't seen before. The animal was photographed (Figure 1) and, not being the subject of her study, released on the bottom. Three years later, the photograph was shown to the first author, who immediately recognized the clam as *P. candida* since several years before he had discovered empty valves of this species in the same general area (Díaz and Borrero, 1995).

On January 26, 2008, two of us (JMD and FG) visited Bahía Concha in order to search for other living specimens of *P. candida*. After almost one hour diving along the shore, we detected a pair of openings slightly protruding from the sandy bottom at 3 m depth. These structures matched the size and shape of the apertures of the large, bifid siphonal tube of *P. candida* (Figure 2). Both apertures closed sphincter-like and retracted slightly into the sediment when we started to dig around them. Indeed, we dug out a specimen of the "living fossil," though not as large as the specimen found three years before. Unfortunately, the anterior part of both valves broke during collecting, but the entire soft parts of the animal were still present; the length of the valves was approximately 70 mm and the siphonal tube was 55 mm long. The specimen was preserved in 100% alcohol and deposited in the marine invertebrate collection at the Universidad de Los Andes in Bogotá (IM–Andes 559).

In regard to the mode of life of *P. candida*, the specimen was positioned nearly vertically in the bottom at a small angle, on its anteroventral margin. This observation agrees completely with the inferred life position of *P. gigantea*

Figure 1. Specimen of *Pholadomya candida* found on November 2004 in Bahia Concha, Colombia.

(J. de C. Sowerby in Fitton, 1836) from the Early Cretaceous of west-central Argentina by Lazo (2007: fig. 8). This author also stated (p. 385) that "in modern *P. candida* the shortness of the (ventral) inhalant siphon relative to the exhalant may not indicate that the animal lay on its back as suggested by Morton (1980: fig. 57). The longer siphon may function as a sort of tube or chimney to discharge waste water well-above the entrance of clean water at the inhalant siphon aperture." Therefore, it seems likely that *P. candida* has a suspension-feeding habit rather than a pedal-feeding system as postulated by Morton (1980) based on the presence of a pedal gape and accessory muscles. A suspension-feeding habit has been commonly suggested in Jurassic and Cretaceous *Pholadomya* species as well (references in Lazo, 2007). The habitat of *P. candida*, at least in Bahía Concha, also suggests a suspension-feeding

Figure 2. Close-up view (from above) of the openings of the siphonal tube of *Pholadomya candida* protruding from the bottom surface.

mode of life. The habitat here is characterized by coarse grain sediments in a shallow setting adjacent to the beach, where water motion caused by incoming waves and drift current is clearly perceptible. This is not the appropriate environment for accumulation of enough detritus on the bottom to guarantee the alimentary requirements of a relatively large, almost sessile, deposit feeder.

The present record is definitive evidence that *Pholadomya candida* is not extinct. Moreover, the specimen collected makes it now possible to undertake genetic sequencing of the only modern representative of an ancient lineage. Comparative molecular sequencing of *P. candida* with other anomalodesmatan species and representatives of additional, presumably related groups may provide not only an insight into the evolution of the other widely differing superfamilies of the Anomalodesmata, but might also reveal clues as to the origin of the Myoida.

ACKNOWLEDGEMENTS

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Research Note

Thiara scabra (O. F. Müller, 1774): The introduction of another Asian freshwater snail into the United States

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The Thiaridae consists of numerous species of non-marine snails in Africa, Asia, Australia, tropical Ameriea, and many island archipelagoes (Morrison, 1954, Glaubrecht, 1999). The family is unusual in that parthenogenetic reproduetion is the normal reproductive mode, which causes each individual to be reproductively isolated from all other individuals. Thus, a single specimen is all that is required to establish a new colony in an appropriate habitat. A recent introduction into Florida freshwater systems is one such species.

Thiara scabra (O. F. Müller, 1774)
The Pagoda Tiara
(Figure 1)

OBSERVATIONS

Identification: The species is recognized by the following eombination of eharaeters (Brandt, 1974). It has a neomelanian operenlum: the nucleus is offset strongly toward the lower columellar margin (Thompson, 2006: fig. 38). The shell is medium-sized, generally up to 20 mm in length, and eonsists of 6–8 whorls remaining in adults. Usually early juvenile whorls are worn away. The shell is pagodiform with strongly shouldered whorls that bear regularly spaced stout spines or knobs. Strongly impressed spiral sculpture is present, which usually is most distinct below the periphery. The ground color is tawny with vertieal, rust-colored flames and blotches alternating with the spines.

Distribution: Widely deployed and locally abundant throughout its range in South and Southeast Asia, South China, the Indo-Australian Archipelago, and western Pacific Islands (Brandt, 1974: 163–164). The senior author has encountered this species on many oceasions in Southeast Asia. It is tolerant to many environmental settings, as is refleeted by its wide distribution. One such setting is eanals in semitropical regions, such as are abundant in South Florida. It is not surprising to us that the snail finally was found there. It is a medium-sized, ornate organism that has potential in the aquarium trade. We suspect that the species was deliberately introduced because of the isolated locality where it is found.

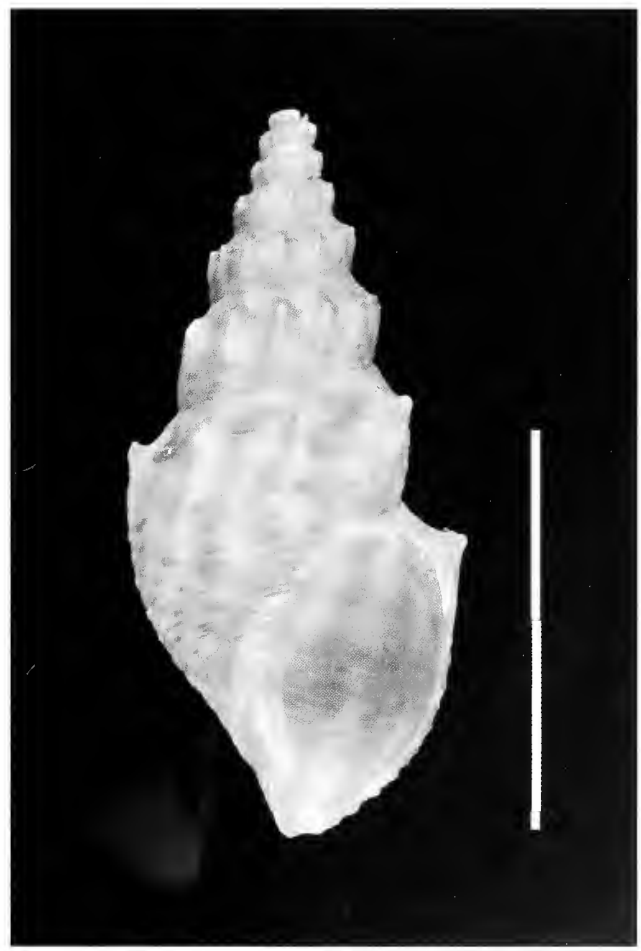

Figure 1. *Thiara scabra* (O. F. Müller, 1774). UF 391616. Scale bar = 1 cm.

Discussion: On September, 2006, specimens were first collected by Todd Ennis and Cheri Hughes of Tetra Tech, Inc. at the Port Mayaca Aquifer Injection well site (UF 391616). The Port Mayaca site is located in the northwest corner of Section 14, Township 40 South, Range 37 East, near the confluence of the L-65 Canal and St. Lucie River (C-44 Canal) in the Town of Port Mayaca, Martin Co., Florida (26°59′17" N, 80°36′22" W). The site is located on a South Florida Water Management District (FWMD)-owned parcel of land adjacent to the S-153 spillway and lock, which conveys water to and from the L-65 Canal and the St. Lucie River. The location is approximately 2,000 feet east of the Herbert Hoover Dike, south of the service access road, and approximately 100 feet west of the intersection with the L-65 canal. Property to the north is under cultivation of sugar cane.

Doug Strom of Water and Air Research Water & Air Research, Inc., Gainesville, Florida, reports to us that a co-worker, Laura Line, collected this snail in the West Palm Beach Canal, 5 kilometers from its junction with Lake Okeechobee on April 10, 2007.

Vernacular Etymology: The vernacular name Pagoda Tiara is taken from Reeve (1860, pl. 26, fig. 182), from a name he proposed for a common form of this species. Tiara comes from the generic name *Thiara*, which is derived from Persian through the Classical Greek, and means a tiara. *Thiara scabra* possess a corona of spines on the shoulder of the whorls reminiscent of a tiara.

ACKNOWLEDGMENTS

We thank Rob Lasley, Florida Museum of Natural History, for photographing Figure 1.

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Erratum

A review of *Typhisopsis* Jousseaume, 1880, and *Typhisala* Jousseaume, 1881 (Gastropoda: Muricidae) of the eastern Pacific (2006)

Typhisopsis carolskoglundae was described by Houart and Hertz (2006: 56, figs. 17–25, 47–49, 59, 63). The holotype from Costa Rica was deposited in SDNHM under registration number 90773. However, there were paratypes of which five were stated as from Boca de la Honda, Panamá at 7°27′ N, 80°51′ W, and deposited in the following institutions: MZUCR (one specimen, registration n° 6153); MNHN (one specimen, registration n° 6991); BM(NH) (1 specimen, registration n° 20050371) and 2 specimens in R. Houart coll. (no registration number).

In a recent article, Villalobos-Rojas et al. (2008) stated: "***carolskoglundae***, *Typhisopsis*, Houart and Hertz, 2006: 56–58, figs. 17–25, 47–49, 59, 63. Type locality: Playas del Coco, Guanacaste, Costa Rica (10°55′53″ N, 85°69′51″ W), 24–37 m depth, on mud bottom. PARATYPE MZUCR-6153 (shell, Figure 5). Boca de la Honda, Veraguas, Panamá (7°27′ N, 80°51′ W), in white sand. Remarks: The coordinates and the collecting locality of this paratype appear to be incorrect since these coordinates plot inland."

After having again contacted the person who found these specimens several years ago, we learned that the published locality was in error, in part due to the label which was written "B. Honda" and which was misinterpreted as "Boca de la Honda". The exact locality where these specimens (now paratypes) were found is not Boca de la Honda, Panamá, but **Bahia Honda, Panamá**, a place situated in the Golfo de Chiriquí, West of Isla Cébaco. The senior author already contacted the institutions where the paratypes were deposited and gave the exact locality data.

Abbreviations: BM(NH): The Natural History Museum, London; MNHN: Muséum national d'Histoire naturelle, Paris; MZUCR: Museo de Zoologia, Universidad de Costa Rica, San José; SDNHM: San Diego Natural History Museum, California, USA.

ACKNOWLEDGMENTS

Thanks to André Vassart (Costa Rica) for his very useful cooperation.

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Notice

THE 2009 R. T. ABBOTT VISITING CURATORSHIP

The Bailey-Matthews Shell Museum is pleased to invite applications for the 2009 R. T. Abbott Visiting Curatorship.

The Curatorship, established originally in accordance with the wishes of the late Dr. R. Tucker Abbott, Founding Director of the Shell Museum, is awarded annually to enable malacologists to visit the museum for a period of one week. Abbott Fellows are expected, by performing collection-based research, to assist with the curation of portions of the Museum's collection and to provide one evening talk for the general public. The Museum collection consists of marine, freshwater, and terrestrial specimens. A large percentage of our holdings have been catalogued through a computerized database management system; part of the catalogue is already available for searches online at: www.shellmuseum.org/collection.html. A substantial portion of the time will be available for research in the collection, but field work in southwest Florida can be arranged. The R. T. Abbott Visiting Curatorship is accompanied by a stipend of $1,500.

Interested malacologists are invited to send a copy of their curriculum vitae, a letter detailing their areas of taxonomic expertise and research objectives, and to provide a tentative subject for their talk. Send materials to:

Dr. José H. Leal, Director
The Bailey-Matthews Shell Museum
P.O. Box 1580
Sanibel, FL 33957
jleal@shellmuseum.org

Applications for the 2009 Visiting Curatorship should be sent electronically to the above e-mail address no later than May 15, 2009, or postmarked by that date if sent by regular mail. The award will be announced by mid-June 2009. Questions about the Visiting Curatorship should be sent to the e-mail address above, or by phone at: (239) 395-2233; fax (239) 395-6706

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NATIONAL
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THE NAUTILUS publishes articles on all aspects of the biology, paleontology, and systematics of mollusks. Manuscripts describing original, unpublished research and review articles will be considered. Brief articles, not exceeding 1000 words, will be published as notes and do not require an abstract. Notices of interest to the malacological community will appear in a notices section.

Manuscripts: Each original manuscript and accompanying illustrations should be submitted to the editor preferably via e-mail or as hardcopy in triplicate.

Text must conform to the dimensions of 8½ × 11-inch paper, double-spaced, and single-column throughout (including literature cited, tables, and figure captions). Authors should follow the general recommendations of *Scientific Style and Format—The CSE Manual for Authors, Editors, and Publishers,* available from the Council of Science Editors at www.councilscienceeditors.org. The first mention of a scientific name in the text should be accompanied by the taxonomic authority, including year. Latinized names and other words to be printed in italics must be underlined; leave other formatting indications to the editor. Metric, not English, units are to be used. Geochronologic modifiers should be capitalized only when units are formally recognized: for instance, use Late Cretaceous but early Miocene. Likewise, only modifiers of formally recognized chronostratigraphic units are capitalized: use Lower Jurassic but upper Oligocene.

The sequence of sections should be title page, abstract, introduction, materials and methods, results, discussion, acknowledgments, literature cited, tables, figure captions, figures. The title page should include the title, author's name(s) and address(es). If corresponding author is not the senior author, please indicate. The abstract should summarize in 250 words or less the scope, main results, and conclusions of the article. Abstracts should be followed by a list of additional key words. All references cited in the text must appear in the Literature Cited section and vice-versa. Please follow a recent issue of THE NAUTILUS for bibliographic style, noting that journal titles must be unabbreviated. Information on plates and figures should be cited only if not included within the pagination of cited work. Tables must be numbered and each placed on a separate page. If in doubt, please follow a recent issue of the journal for sequence of sections and other style requirements.

Illustrations: Illustrations are rendered either at full-page width (maximum width 17 cm) or column width (maximum width 8.2 cm). Please take these dimensions into consideration when preparing illustrations. Page-width illustrations ideally should span the entire width of printed page (17 cm). "Tall" page-width illustrations should be avoided, square or "landscape" formats work better. Please design plates accordingly, such that there will be enough space left at the bottom of printed page for plate caption. (Digital technology has made this task much easier.)

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A new species of freshwater mussel, *Anodonta hartfieldorum* (Bivalvia: Unionidae), from the Gulf Coastal Plain drainages of Alabama, Florida, Louisiana, and Mississippi, USA

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ABSTRACT

A new species of unionid mussel, *Anodonta hartfieldorum*, is described from Coastal Plain streams of the eastern Gulf of Mexico drainages. It occurs in the Pearl River in Louisiana and Mississippi, Pascagoula River in Mississippi, Tombigbee River in Mississippi and possibly Alabama, Tensaw River in Alabama and the Escambia River drainage in Alabama and Florida. Based on shell morphology and presence of very thin green rays, it belongs to a species group within the genus *Anodonta* which includes *A. couperiana, A. heardi, A. implicata,* and *A. suborbiculata. Anodonta hartfieldorum* appears to be most closely related to *A. suborbiculata*, but differs in several aspects of shell morphology. *Anodonta suborbiculata* is widespread in the Mississippi Basin and has been widely introduced outside its native range. *Anodonta hartfieldorum* occurs in floodplain sloughs and oxbow lakes in silty sand to mud sediments. Its conservation status is unknown as its typical habitat is underrepresented in most mussel sampling programs.

Additional keywords: Cypress Floater, new species, taxonomy, conservation, Alabama, Florida, Louisiana, Mississippi

INTRODUCTION

Southeastern United States Unionidae have received considerable attention during the past half century. Thus, their taxonomy is well understood relative to some other groups of aquatic invertebrates (e.g. crayfishes) (Taylor et al., 2007). However, there are remaining undescribed unionid species (Williams et al., 2008). One undescribed species of *Anodonta* Lamarck, 1799, was first recognized in the Pascagoula River during the 1980s by Paul Hartfield, U.S. Fish and Wildlife Service, Jackson, Mississippi. Subsequent surveys revealed the presence of this species in Gulf Coast drainages from the Pearl River east to the Escambia River (Vidrine, 1993; Williams et al., 2008).

The genus *Anodonta*, as presently conceived, occurs in most of the Nearctic and Palearctic regions. The type species, *Anodonta cygnea* Linnaeus, 1758, is from western Europe. In North America, *Anodonta* occurs from Alaska and Canada to Mexico. Turgeon et al. (1998) recognized ten species in the genus. The number of *Anodonta* species worldwide is not clear due to poor delineation of taxa and varying interpretations of the species concept (e.g. Mock et al., 2004). Ortmann (1912) observed that "in Europe the species-making in this group has gone beyond all the bounds of reason." *Anodonta* Lamarck, 1799, is a *nomen conservatum* (ICZN, 1926: Opinion 94; 1959: Opinion 561). Hoeh (1990) used morphological and allozyme data to produce a phylogeny that divided North American *Anodonta*, along with the European type species *A. cygnea*, into three clades: *Anodonta*; *Pyganodon* Crosse and Fischer *in* Fischer and Crosse, 1894; and *Utterbackia* Baker, 1927. The latter two were elevated from subgeneric to generic status by Hoeh (1990).

MATERIALS AND METHODS

Comparative material of *Anodonta couperiana* Lea, 1840, *Anodonta hartfieldorum*, *Anodonta heardi* Gordon and Hoeh, 1995, and *Anodonta suborbiculata* Say, 1831, from several museums was utilized in this description. These institutions include Florida Museum of Natural History, University of Florida (UF), Gainesville, Florida; Mississippi Museum of Natural Science (MMNS), Jackson, Mississippi; Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts; North Carolina State Museum of Natural Sciences (NCSM), Raleigh, North Carolina; Ohio State University Museum (OSUM), Columbus, Ohio; and National Museum of Natural History, Smithsonian Institution (USNM), Washington, DC. Additional material from the personal collection of Robert G. Howells, Kerrville, Texas, was also examined.

Shell measurements were made to the nearest millimeter using digital calipers and included total length,

height, width, hinge line length, and distance from umbo to posterior shell terminus. Total length is defined as distance between anterior and posterior margins, measured parallel to the hinge line. Shell height is distance between dorsal and ventral margins, measured near the midpoint of the hinge line, perpendicular to shell length. Shell width is maximum distance between the outer surfaces of the paired valves. Hinge line length is distance from its anterior terminus to the anterior end of the ligamental notch. Distance from the center of the umbo to the posterior shell terminus was measured in a straight line. The angle between dorsal margin and posterior margin was measured to the nearest five degrees using a protractor (Figure 1). Shell measurement data were analyzed using Excel spreadsheet scatter plots with linear regression. An ANOVA on the angle measurements was performed on the three species using SPSS v16.0. A post-hoc comparison using Tukey's test was performed to determine the significantly different groups. Gross anatomy of soft tissues was described from fresh and relaxed specimens fixed in 10% formalin and transferred to 70% ethanol. The anatomical descriptions follow methods described in Williams et al. (2008).

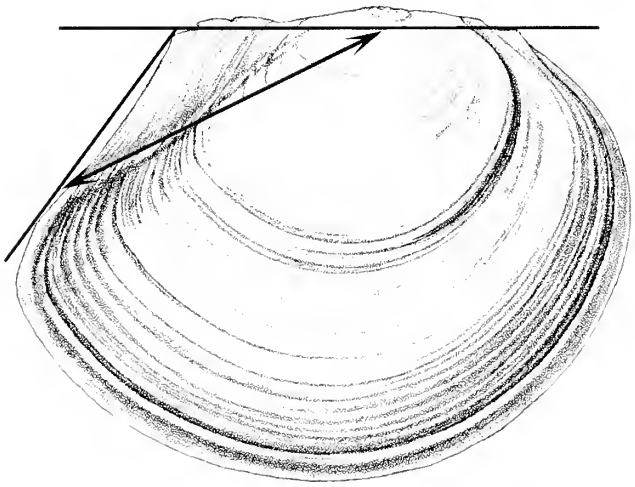


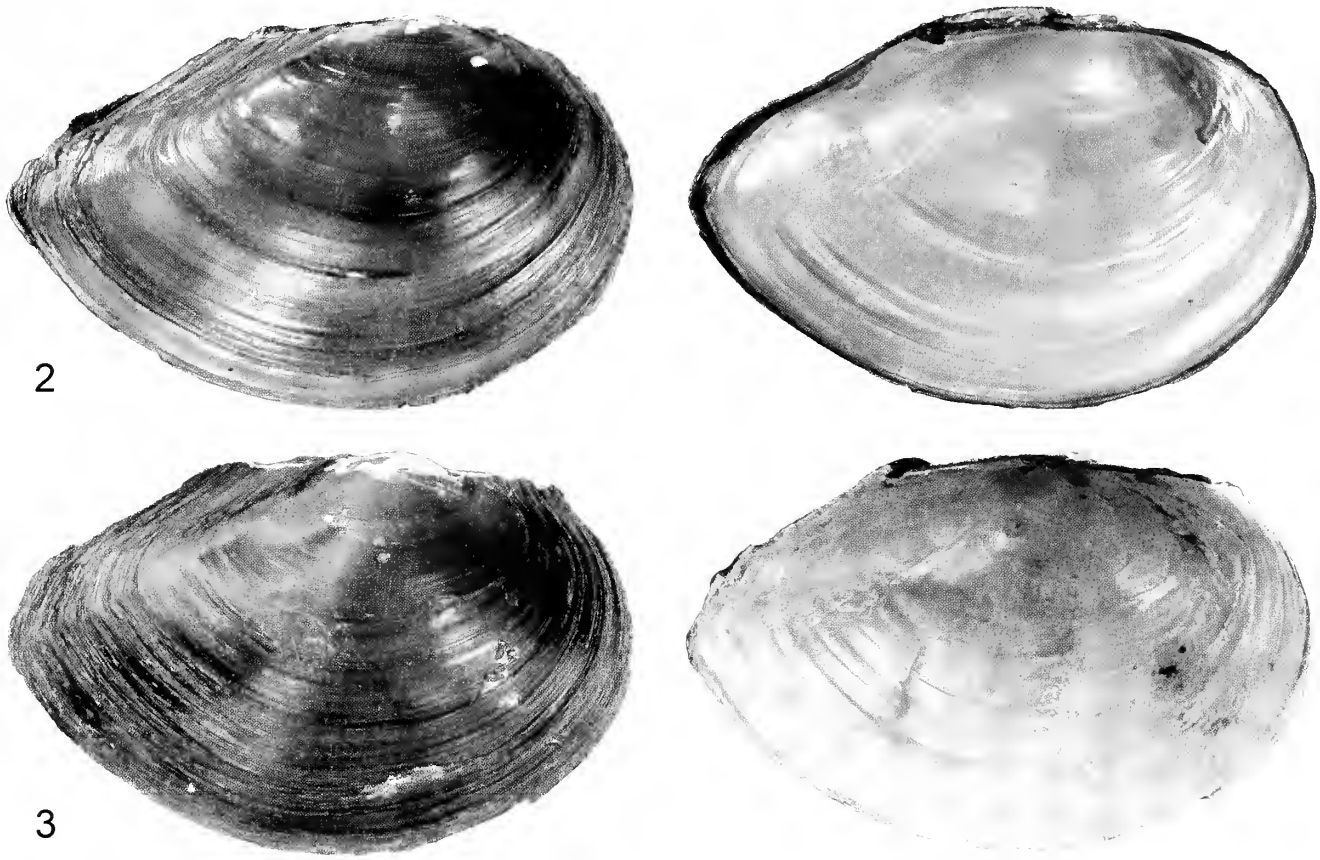
Figure 1. Illustration of how the angle between dorsal margin and posterior margin was measured. Image of *Anodonta suborbiculata* modified from Burch (1975).

Anodonta hartfieldorum new species
Cypress Floater
(Figures 2, 3)

Diagnosis: *Anodonta hartfieldorum* is distinguished from other unionid species by a combination of the following characteristics: thin, compressed to inflated shell, elliptical to oval outline, ventral margin rounded; angle between dorsal margin and posterior margin usually 140° to 150°; hinge teeth absent; periostracum smooth, tawny to olive or brown, typically with very thin green rays; umbo only slightly elevated above hinge line; umbo sculpture in the form of parallel bars in adults; inner lamellae of inner gills connected to visceral mass only anteriorly; supra-anal aperture small, separated from excurrent aperture by wide mantle bridge (may be longer than either of the two apertures); outer gills marsupial; marsupium occupying entire gill, well padded when gravid; secondary water tubes present in gravid marsupia; glochidium with styliform hooks.

Description: Length to 120 mm; shell thin; moderately inflated; outline oval; posterior margin narrowly rounded to bluntly pointed; angle between dorsal margin and posterior margin 140° to 155° (mean = 146°) (Table 1); anterior margin broadly rounded; dorsal margin straight; ventral margin convex; posterior ridge low, rounded; posterior slope moderately steep, slightly concave, occasionally extending into a very low dorsal wing; umbo broad, moderately inflated, barely elevated above hinge line; umbo sculpture nodulous ridges in young and parallel bars in adults; periostracum tawny to olive or brown, typically with very thin, olive to greenish brown rays that often are obscure in adults. Pseudocardinal and lateral teeth absent; umbo cavity wide, shallow; nacre white, sometimes with salmon tint in umbo cavity (Figures 2, 3).

In life the mantle is creamy-white to tan or golden-tan, may be dull-orange external to pallial line, mantle outside of apertures dull-orange to grayish-brown; visceral mass creamy-white to tan, may be dull-orange adjacent to foot; foot dull-orange to creamy-white or tan. Gills gold to tan or brown; dorsal margin sinuous to concave, ventral margin convex; gill length 57–69% of shell length; gill height 31–53% of gill length; outer gill height 79–100% of inner gill height, outer gill height may be greater than inner gill height in gravid individuals; inner lamellae of inner gills only connected to visceral mass anteriorly. Outer gills marsupial; glochidia held across gill length; well padded when gravid; light-brown to brownish-orange. Labial palps tan, may have golden cast; straight to concave dorsally, convex ventrally, bluntly pointed distally; palp length 21–40% of gill length; palp height 46–67% of palp length; distal 27–58% of palps bifurcate. Incurrent aperture usually longer than excurrent and supra-anal apertures; supra-anal and incurrent apertures occasionally of similar length. Incurrent aperture length 8–12% of shell length; creamy-white to dull-orange within, sometimes grayish or rusty-brown basal to papillae; papillae in 2–3 rows, inner row usually larger, simple, short, thick; papillae tan to dull-orange, larger papillae often with black edges basally. Excurrent aperture length 5–8% of shell length; creamy-white to dull-orange within, marginal color band rusty-tan to dull-orange with black lines perpendicular to margin, generally with some lines converging proximally, some individuals with lines interconnected to form an irregular reticulated pattern; excurrent aperture margin smooth, may undulate. Supra-anal aperture length usually 5–8% of shell length, occasionally to 16% of shell length; creamy-white to tan within, usually without marginal coloration, occasionally with a thin, irregular tan band; supra-anal aperture margin smooth; mantle bridge separating supra-anal and excurrent apertures usu-



Figures 2, 3. *Anodonta hartfieldorum*. **2.** Holotype UF 375595, length 112 mm. Fish Lake, oxbow off Pascagoula River, 1 air mile [1.6 air kilometers] southeast of Highway 614 bridge, southwest of Wade, 30.6016°N; 88.6233°W, Jackson County, Mississippi, 27 Oct. 2000. © Richard T. Bryant. **3.** Non-type specimen UF 358657, length 114 mm. Slough and gravel pits adjacent to Escambia River, Mystic Springs boat ramp, 1 mile [1.6 kilometer] southeast of McDavid, Escambia County, Florida, 30.92656°N; 87.28597°W, 20 Sep. 1999. © Richard T. Bryant.

ally imperforate, of variable length, 67–480% of supra-anal length, occasional individuals with a short secondary mantle bridge anterior to primary bridge.

Minor soft anatomy differences were noted between *Anodonta hartfieldorum* specimens collected from the Pascagoula and Escambia River systems. Incurrent apertures of Pascagoula individuals (8–10% of shell length) were shorter than those of Escambia individuals (10–12% of shell length). Length of the mantle bridge separating excurrent and supra-anal apertures varied more widely in Pascagoula individuals (67–480% of supra-anal length) than in Escambia individuals (90–420% of supra-anal length). Conversely, wider variation in labial palp size was observed in Escambia individuals (palp length/gill length 21–40%; palp height/palp length 47–67%) than Pascagoula individuals (palp length/gill length 25–29%; palp height/palp length 54–57%). Gill height in relation to gill length was greater in Escambia individuals (40–53%) than in Pascagoula individuals (31–38%). These differences are considered slight and could be an artifact of modest sample sizes (n = 6 from each of the two drainages), so further comparisons are needed. No material from the Pearl River drainage or Mobile Basin was available for comparison.

Table 1. Frequency distribution of the angle measurement between dorsal margin and posterior margin of *Anodonta hartfieldorum*, *A. heardi* and *A. suborbiculata*. *Anodonta suborbiculata* was significantly different ($p < 0.001$) from the other two species. There was no significant difference among the other two species ($p = 0.534$).

Species	Angle between dorsal margin and posterior margin											
	115°	120°	125°	130°	135°	140°	145°	150°	155°	160°	N	Mean
A. hartfieldorum						18	25	19	7		69	146
A. heardi				1	1	1	5	6	2	2	18	147
A. suborbiculata	3	17	38	34	25	8	2				127	129

Type Material: Holotype: UF 375595, length 112 mm, Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), Jackson County, Mississippi, 27 Oct. 2000. **Paratypes: Pascagoula River Drainage**: **Mississippi: Jackson County:** MCZ 361689, length 89–107 mm (3 dry shells), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000. MMNS 6973, length 60–101 mm (6 dry shells), Pascagoula River at mouth of Dead River Lake (30.59236°N; 88.59761°W), 27 Aug. 1986. NCSM 29799, length 58–78 mm (2 alcohol preserved), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000. NCSM 28212, length 57–101 mm (13 alcohol preserved), Dead River Lake (mouth) off Pascagoula River, [6 air km SSW center of Wade] (30.5944°N; 88.5979°W), 27 Aug. 1986. OSUM 80078, length 88–106 mm (3 dry shells), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000. UF 428535 (6 in 95% alcohol preserved), Pascagoula River at Paper Mill Camp (30.63228°N; 88.65240°W), 21 Aug. 2008. UF 428536 (4 in 70% alcohol preserved), Pascagoula River at Paper Mill Camp (30.63228°N; 88.65240°W), 21 Aug. 2008. UF 428544, length 53–120 mm (9.5 dry shells), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000. UMMZ 302000, length 90–105 mm (3 dry shells), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000. USNM 1124163, length 79–99 mm (4 dry shells), Fish Lake, oxbow off Pascagoula River, 1 air mi. [1.6 air km] SE of Hwy 614 bridge, SW of Wade (30.6016°N; 88.6233°W), 27 Oct. 2000.

Other Material Examined: Escambia River Drainage: Alabama: Covington County: NCSM 45095 (16 alcohol preserved), Gantt Reservoir, Conecuh River, CR 86 [Dunn's Bridge Road], [3.2 air km NE center of Gantt] (31.42573°N; 86.4576°W), 5 Nov. 2006. NCSM 45145 (1 dry shell), Point A Reservoir [Conecuh River], SW corner, 1.77 km NW from intersection of CR 70 and US 84, (31.35953°N; 86.51628°W), 8 Nov. 2005; **Alabama: Escambia County:** UF 375317 (1 alcohol preserved), Old Faulkner Lake, oxbow lake of Conecuh River, 2 air mi. [3.2 air km] SE of Pollard, 0.5 air mi. [0.8 air km] N of Florida state line, 29 June 1995; **Florida: Escambia County:** UF 358657 (6 dry shells), slough and gravel pits adjacent to Escambia River, at Mystic Springs boat ramp, 1 mi. [1.6 km] SE of McDavid (30.92656°N; 87.28597°W), 20 Sep. 1999. UF 376605 (5 dry shells), slough and gravel pits adjacent to Escambia River, at Mystic Springs boat ramp, 1 mi. [1.6 km] SE of McDavid, 5 July 1992. UF 428537 (3 in 70% alcohol preserved), Escambia River at Bluff Springs boat ramp (30.92675°N; 87.28647°W), 19 Sep. 2007. UF 428538 (6 in 95% alcohol preserved), Escambia River at Bluff Springs boat ramp (30.92675°N; 87.28647°W), 19 Sep. 2007. NCSM 28251 (16 alcohol preserved), Escambia River, abandoned gravel pits adjacent to Mystic Springs boat ramp, [point estimated 1.6 air km SSE center of] McDavid (30.85559°N; 87.31266°W), 9 Aug. 1992.

Mobile Tensaw River Drainage: Alabama Baldwin County: UF uncataloged (2 alcohol preserved), slough off Tensaw Lake about 1 air mi. [1.6 air km] SSW of Hubbard Fish Camp and Landing (31.049097°N; 87.871753°W), 18 Sep. 1999. These specimens were misplaced during a transfer from U.S. Geological Survey to the Florida Museum of Natural History.

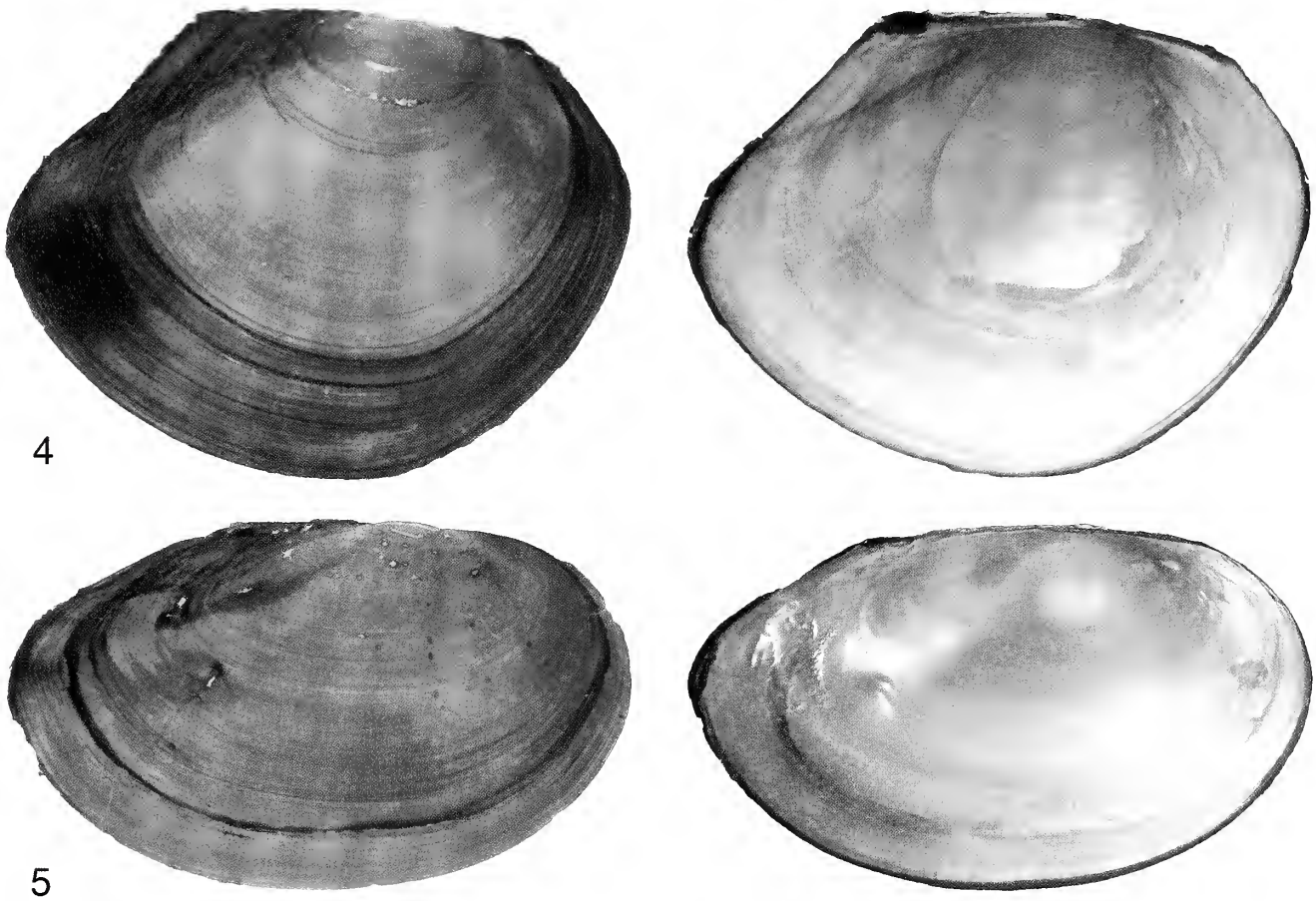
Pascagoula River Drainage: Mississippi: George County: MMNS 5503 (3 dry shells), McCrea Dead River E of Dale (30.83302°N; 88.74750°W), 29 May 2000.

Pearl River Drainage: Louisiana: St. Tammany Parish: MMNS 6444 (1 dry shell), Pearl River, Mississippi and Louisiana state line, at Walkiah Bluff, mouth of slough about 0.5 mi. [0.8 km] upstream of boat ramp, 18 Sep. 1986. Specimen was not examined; record based on a personal communication with Bob Jones, MMNS; **Mississippi: Marion County:** MMNS 2168 (1 dry shell), Pearl River, in vicinity of Columbia, 24 Apr. 1986.

Comparison with Similar Species: *Anodonta hartfieldorum* shells resemble those of *A. suborbiculata* (Figure 4) but are less round and usually more inflated, with a more inflated umbo that is elevated slightly above the hinge line. It also may resemble *Pyganodon grandis*, but that species has a much more inflated umbo that is considerably elevated above the hinge line. *Anodonta hartfieldorum* may vaguely resemble *Utterbackia imbecillis* and *Utterbackia peggyae* Johnson, 1965, but those species are more elongate and their umbos are not elevated above the hinge line. *Anodonta hartfieldorum* is similar in shell morphology to *A. heardi* (Figure 5), but the two species are allopatric, with *A. heardi* occurring only in the Apalachicola Basin and eastward in the Ochlockonee River (Gordon and Hoeh, 1995; Brim Box and Williams, 2000).

Shell proportions of *Anodonta hartfieldorum* differ from those of *A. suborbiculata* and *A. heardi*. The most notable differences are in the relative proportions of shell height and length, as well as the angle between dorsal margin and posterior margin. *Anodonta hartfieldorum* shell height, relative to length, is greater than that of *A. heardi* but less than that of *A. suborbiculata* (Figure 6). The angle between dorsal margin and posterior margin is about equal in *A. hartfieldorum* (mean = 146°, N = 68) and *A. heardi* (mean = 147°, N = 19) but is greater than that of *A. suborbiculata* (mean = 129°, N = 127). Frequency distributions of these angles are presented in Table 1.

Distribution: *Anodonta hartfieldorum* occurs from the Escambia River drainage in Florida and Alabama west to the Pearl River drainage in Louisiana and Mississippi (Figure 7). It is known from the Escambia River,



Figures 4, 5. *Anodonta* species. **4.** *A. suborbiculata.* UF 376151, length 124 mm. Coosa River, Weiss Reservoir, mouth of Big Cedar Creek, about 2 air miles [3.2 air kilometers] east of Alabama and Georgia state line, Coosa River Mile 258, 34.18473°N; 85.40549°W, Floyd County, Georgia, 20 Aug. 1997. © Richard T. Bryant. **5.** *A. heardi.* UF 358656, length 113 mm. Harrison Creek, north side of first 180° bend, above confluence of Brothers River, 29.873019°N; 85.037933°W, Franklin County, Florida, 7 Sep. 1991. © Richard T. Bryant.

Escambia and Santa Rosa counties, Florida, upstream to Gantt Reservoir, on Conecuh River, Covington County, Alabama. In the Mobile Basin, it is known from the Tombigbee River drainage in Lowndes County, Mississippi, and a single site on the Tensaw River, Baldwin County, Alabama. *Anodonta hartfieldorum* is found in lower reaches of the Pascagoula River drainage in George and Jackson counties, Mississippi, and has been reported from Pearl River in Mississippi (Vidrine, 1993; Jones et al., 2005) and in Louisiana where the Pearl River forms a common border between the two states.

Habitat and Biology: *Anodonta hartfieldorum* occurs in water with little or no current such as oxbow lakes and sloughs. It has colonized Gantt and Point A reservoirs. Substrates in these habitats are typically composed of mud or muddy sand, often with detritus.

Anodonta hartfieldorum is a long-term brooder, presumably gravid from late summer or autumn to the following spring or summer. Gravid individuals brooding mature glochidia have been observed in late October and early November in Pascagoula River and Gantt Reservoir, Conecuh River, respectively. Glochidial hosts of this species are unknown.

Discussion: *Anodonta hartfieldorum* appears to belong to a species group that includes *A. suborbiculata*, *A. conperiana*, *A. heardi*, and *Anodonta implicata* Say, 1829. A common morphological feature shared among these species is umbo sculpture that consists of parallel bars in adults. Juveniles of this group typically have fine green rays radiating from the umbo, but this feature is often obscure in adults with a darker periostracum. Molecular genetic data supports the relationship of this group, which is confined to the eastern United States (Zanatta et al., 2007). *Anodonta suborbiculata* is native to the Mississippi Basin and some central Gulf Coast drainages, *A. hartfieldorum* and *A. heardi* occur in eastern Gulf Coast drainages, and *A. conperiana* and *A. implicata* in Atlantic Coast drainages. There are populations of *A. suborbiculata* in the Brazos, Neches, and Sabine River drainages, east Texas, that differ somewhat in shell morphology (Howells et al., 1996). They are slightly more inflated than typical *A. suborbiculata*.

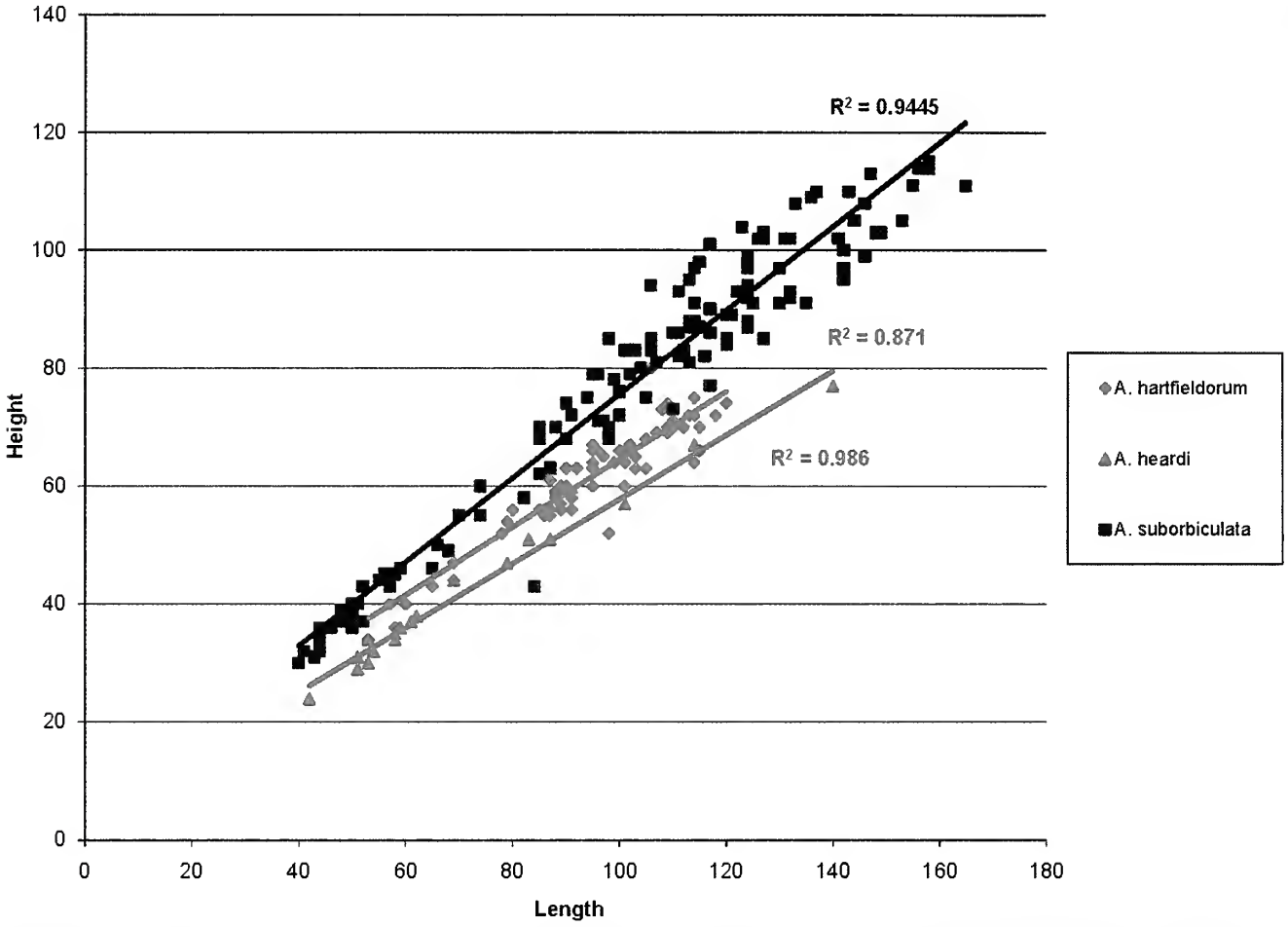


Figure 6. Scatter plot of the relationship between shell height and shell length (mm) in *Anodonta hartfieldorum*, *A. heardi*, and *A. suborbiculata*.

However, additional research is needed to resolve the relationships of these populations.

There have been several reports of *Anodonta suborbiculata* in the Escambia River drainage in Alabama and Florida. The first known Escambia drainage specimen of *Anodonta* was collected in 1917 by C. A. Burke, from Chumuckla Springs, Santa Rosa County, Florida. This specimen (UMMZ 101375) was reported as *A. suborbiculata* by Butler (1990) on the basis of a personal communication from William H. Heard, but it is most likely based on *A. hartfieldorum* (this specimen could not be located to confirm identification). Clench and Turner (1956) did not encounter *A. suborbiculata* in their survey of freshwater mollusks of Florida and southern Alabama. The report of *A. suborbiculata* from Gantt Lake, Covington County, Alabama (MCZ 267518), by Johnson (1969) is based on *A. hartfieldorum*. In the identification manual of freshwater clams of Florida, Heard (1979) reported and illustrated *A. suborbiculata* from the Escambia River drainage but locality data for the illustrated specimen were not given. A single juvenile (48 mm; UF 134930) *A. suborbiculata* from the Escambia River, Florida, was reported and illustrated by Williams and Butler (1994). *Anodonta suborbiculata* appears to be a recent colonizer of the Escambia drainage, with the first confirmed records from the 1980s. The two species occur syntopically in Gantt and Point A reservoirs, Conecuh River, Alabama.

The natural oxbow and slough habitats of *Anodonta hartfieldorum* are often overlooked or avoided during mussel surveys, and these habitats have been greatly reduced due to channelization and impoundment of large rivers. These factors probably contributed to the dearth of records and museum material. A systematic survey of Gulf Coast floodplain lakes and reservoirs is required to determine the current conservation status of *A. hartfieldorum*. Additional comparative analyses of soft anatomy and molecular genetics are also needed to firmly resolve taxonomic relationships within the genus *Anodonta*.

Conservation Status: The fact that *Anodonta hartfieldorum* has not been previously recognized has precluded its inclusion in conservation status reviews. However, this species does not appear to be imminently imperiled. It can be locally abundant, but may have declined in some floodplain lakes and sloughs that have been negatively

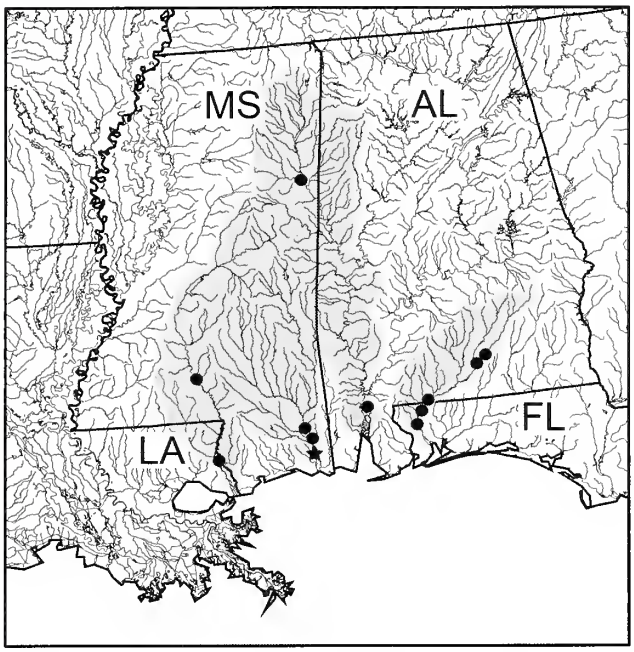


Figure 7. Known range of *Anodonta hartfieldorum* (shaded area) in Alabama, Florida, Louisiana and Mississippi. Solid circles represent specific localities of *A. hartfieldorum*. Type locality of *A. hartfieldorum* is indicated by the star.

affected by channel incision following channelization of adjacent rivers. *Anodonta hartfieldorum* conservation status will remain unresolved until a systematic survey of appropriate habitat is conducted.

Etymology: The species name *hartfieldorum* is in honor of Paul D. and Elizabeth A. Hartfield in recognition of their significant contributions to conservation and natural history in the southeastern United States. Paul is a biologist in the endangered species program, U.S. Fish and Wildlife Service, Jackson, Mississippi, field office, and has been instrumental in protection and recovery of aquatic species. Elizabeth (Libby) is director of the Mississippi Museum of Natural Science, Jackson, Mississippi, where she presided over the enhancement of the institution, which is now one of the premier museums in the southeast. She has also played an integral role in conservation and environmental education. The common name, Cypress Floater, is in reference to the Cypress tree which is common along the flood plain sloughs and backwater oxbow lakes where *Anodonta hartfieldorum* is found.

Comparative Material Examined:

Anodonta heardi

Apalachicola River Drainage: Florida: Franklin County: UF 358656 (1 dry shell), Harrison Creek, [tributary of Apalachicola River] at first 180° turn, N side of bend (29.873019°N; 85.037933°W), 7 Sep. 1991.

Florida: Gulf County: NCSM 30334 (1 alcohol preserved), Chipola River, Florida Hwy 22 [CR 22/Lakegrove Road], [2.8 air km NE] of Wewahitchka (30.12766°N; 85.17638°W), 6 Aug. 1988. UF 375520 (1 dry shell), Apalachicola River at river mile 45.3, at the Wewahitchka Boat Ramp, about 5 air mi. [8 air km] NE of Wewahitchka, 1 Sep. 1999. UF 428532 (11 dry shells), Apalachicola River Mile 46.8, along right descending bank of river (30.1819°N; 85.1344°W), 7 Aug. 2006.

Florida: Jackson County: UF 1915 (1 dry shell), Tanvat Pond, 3 mi. [4.8 km] N of Sneads, 1 Apr. 1955.

Florida: Leon County: MCZ 267515 (1 dry shell), Ochlockonee River at US Hwy 27, 11 mi. [17.7 km] NW of Tallahassee.

Florida: Liberty County: UF 381286 (1 dry shell), Florida River, from downstream near SW edge of Acorn Lake to point downstream of head of feeder slough into Everett Slough, 4 June 2002.

Georgia: Crisp County: NCSM 28259 (1 alcohol preserved), Lake Blackshear, 0.3 mi. [0.5 km] S of US 280 bridge at edge of Georgia Veteran's Memorial State Park, [point estimated 6.2 air km E center of Cobb] (31.9624°N; 83.9224°W), Sep. 1992. UF 376024 (2 dry shells), Lake Blackshear, US Hwy 280 crossing (E side) middle of Lake Bridge, June 1995.

Ochlockonee River Drainage: Florida: Leon County: UF 370608 (4 alcohol preserved), Ochlockonee River about 3.5 air mi. [5.6 air km] S [SW] of Rt. 20 bridge (30.34804°N; 84.69356°W), 19 July 1993.

Anodonta suborbiculata

Arkansas River Drainage: Arkansas: Crawford County: USNM 124422 (2 dry shells), [Arkansas River,] Van Buren.

Atchafalaya River Drainage: Louisiana: St. Martin Parish: OSUM 76142 (1 dry shell), 4 mi. [6.4 km] SE of Henderson [6.4 km SE of Henderson, 4.8 km W of Butte La Rose] (30.28140°N; 91.73600°W), 27 Sep. 1975.

Escambia River Drainage: Alabama: Covington County: MCZ 267518 (1 dry shell), Clearview, on Conecuh River, Gantt Lake at US Hwy 29. NCSM 35282 (1 alcohol preserved), Point A Reservoir, [point estimated 2.3 air km NE center of River Falls] (31.36796°N; 86.52074°W), 2005. NCSM 48025 (3 dry shells), Point A Reservoir [Conecuh River], SW corner, 1.77 km NW from intersection of CR 70 and US 84, [2.3 air km NE center of River Falls] (31.35953°N; 86.51628°W), 8 Nov. 2005. UF 375318 (1 alcohol preserved) Patsaliga Creek, slough on impounded lower end, about 0.7 air mi. [1.1 air km] N of CR 59 bridge (31.382518°N; 86.522834°W), 24 July 1995.

Florida: Escambia County: UF 134930 (1 alcohol preserved) Escambia River, Rt. 4 crossing, 2.8 km E of Century, 13 km NNE of McDavid, 7.8 km W of Jay, 3 June 1998.

Mississippi River Drainage: Illinois: Carroll County: UF 225860 (4 dry shells), Thomson Lake.

Tennessee: Shelby County: MCZ 152833 (7 dry shells), Mississippi River, Presidents Island, near Memphis.

Mobile Basin Drainage: Alabama: Cherokee County: UF 374082 (1 dry shell), Coosa River at island, about 0.8 mi. [1.3 km] upstream of Hwy 20 bridge (Garrett Bridge), 7 Aug. 2000. UF 374282 (3 dry shells), Coosa River at Large Island, about 1 mi. [1.6 km] downstream from Maple Grove, 7 Aug. 2000.

Alabama: Monroe County: UF 374748 (10 dry shells), slough off Alabama River, about 1 mi. [1.6 km] upstream of Claiborne Lock & Dam, on west bank, 17 Sep. 1999.

Alabama: Tallapoosa County: UF 376505 (2 dry shells), Lake Martin at Wind Creek State Park, about 6 mi. [9.7 km] S of Alexander City, 28 Jan. 2004.

Alabama: Walker County: OSUM 58673 (1.5 dry shells), Bullbarn Creek, [6.1 km S of Jasper] (33.77000°N; 87.25888°W), 14 Aug. 1993. OSUM 59644 (1 dry shell), Bullbarn Creek, [6.1 km S of Jasper] (33.77000°N; 87.25888°W), 24 Feb. 1997.

Alabama: Wilcox County: UF 244013 (2 dry shells), Millers Ferry, 9 mi. [14.5 km] NW of Camden, 200 m N of Rt. 28 bridge over Alabama River, East Bank Park, 24 Sep. 1988. UF 374742 (1 dry shell), slough off Alabama River, about 1 mi. [1.6 km] upstream of Claiborne Lock & Dam, on west bank, 17 Sep. 1999. UF 376590 (1 dry shell), impoundment of Alabama River, East Bank Park at Millers Ferry, just NE of Hwy 28 bridge, about 7 mi. [11.3 km] W of Camden, 10 Sep. 1988. UF 376593 (1 dry shell), Coosa River at Large Island, about 1 mi. [1.6 km] downstream from Maple Grove.

Georgia: Floyd County: UF 376151 (1 dry shell), Coosa River (Weiss Reservoir), at mouth of Big Cedar Creek, about 2 air mi. [3.2 air km] due E of Alabama and Georgia state line (Coosa River Mile 258) (34.18473°N; 85.40549°W), 20 Aug. 1997.

Mississippi: Prentiss County: MMNS 9072 (2 dry shells), Tombigbee River, borrow pits at Natchez Trace at Brown Bottom, 12 mi. [19.3 km] ESE of Baldwin (34.46835°N; 88.42968°W), 28 Oct. 1999.

Neches River Drainage: Texas: Nacogdoches County: NCSM 30546 (3 dry shells), Sam Rayburn Reservoir, Shirley Creek Park, [point estimated at end of CR 496 (Sowell Bridge Road) in park, 9.7 air km WNW of Broaddus] (31.31503°N; 94.37306°W), 12 Dec. 1995.

Texas: San Augustine County: MMNS 7477 (2 dry shells), Sam Rayburn Reservoir on the Angelina River, 5 Nov. 1995. Robert G. Howells (6 dry shells, 2 alcohol preserved), Sam Rayburn Reservoir, Ayish Creek arm at CR 2923, 12 Dec. 1995.

Texas: Tyler County: Robert G. Howells (1 dry shell), B.A. Steinhagen Reservoir, 29 Dec. 1993.

Ouachita River Drainage: Arkansas: Clark County: UF 64057 (3 dry shells), Old River, Arkadelphia.

Pearl River Drainage: Mississippi: Madison County: MMNS 6263 (1 alcohol preserved), Pearl River, left ascending bank, 0.5 mi. [0.8 km] below Lowhead Dam, above Coal Bluff Water Park (32.61559°N; 89.75558°W), 1 Oct. 1987.

Mississippi: Pearl River County: MMNS 6444 (2 dry shells), Pearl River at Walkiah Bluff, mouth of oxbow 0.5 mi. [0.8 km] upstream from boat launch (30.56513°N; 89.79114°W), 18 Sep. 1986.

Red River Drainage: Louisiana: Bienville Parish: USNM 119969 (1 dry shell), Mount Lebanon.

Louisiana: DeSoto Parish: USNM 133381 (4 dry shells), Frierson Mill.

Louisiana: Rapides Parish: USNM 86699 (1 valve of shell), Red River, Alexandria.

Louisiana: Webster Parish: OSUM 76518 (4 dry shells), Cypress Swamp, [6.7 km E of Doyline, 3.5 km W of Sibley] (32.53757° N; 93.33049° W).

Texas: Marion County: NCSM 33214 (2 dry shells), Lake O' The Pines (Big Cypress Bayou), [point estimated 9 air km WSW center of Kellyville] (32.81322°N; 94.69791°W), 9 July 1996.

Texas: Camp/Titus counties: Robert G. Howells (5 dry shells), Bob Sandlin Reservoir, 10 July 1996.

Tennessee River Drainage: Alabama: Lauderdale County: NCSM 43448 (16 dry shells), Pickwick Lake, Tennessee River, behind small islands 8.8 air km E [center] of Waterloo [Wright Quad] (34.89613°N; 87.96736°W), 6 Feb. 2009. NCSM 43449 (4 dry shells), Pickwick Lake, Second Creek Embayment, 2.9 km NE of Waterloo [Waterloo Quad] (34.93369°N; 88.03724°W), 6 Feb. 2009. UF 294002 (1 dry shell), Elk River, Wheeler Lake, above Hwy 72 bridge, 1 mi. [1.6 km] E of Rogersville, 1 mi. [1.6 km] above confluence with Tennessee River, above launch ramp, 23 Oct. 1998.

Alabama: Limestone County: NCSM 6187 (2 dry shells), Tennessee River Mile 306, Decatur Boat Harbor, [point estimated 1.8 air km E center of Decatur] (34.60472°N; 86.9625°W), 2 Feb. 2000. NCSM 30439 (4 dry shells), Elk River backwaters, [point estimated 5.2 air km NW center of Cartwright] (34.90416°N; 87.10517°W), 7 Nov. 1976.

Alabama: Madison County: NCSM 33215 (2 dry shell), Redstone Arsenal, unnamed tributary that flows into Tennessee River, 22–23 July 1993.

Mississippi: Tishomingo County: UF 376595 (1 dry shell), Yellow Creek downstream of junction with Pickwick Lake on Mississippi Hwy 25, about 1 mi. [1.6 km] SE of North Crossroads, 1 Mar. 1973.

Tennessee: Humphreys County: NCSM 6710 (2 dry shells), Tennessee River at Cuba Landing, I-40 crossing, [point estimated 10.7 air km NE center of Sugar Tree] (35.87826°N; 87.93317°W), 5 Feb. 1977.

Tennessee: Meigs County: UF 365491 (2 dry shells), Sugar Creek embayment of Hiwassee River, Chickamauga Reservoir on CR 306, about 2 mi. [3.2 km] E of junction 306 and 58, 21 Feb. 1998.

Trinity River Drainage: **Texas: Liberty County:** MCZ 227966 (2 dry shells), Wards Prairie Lake near Romayor (30.40965°N; 94.78743°W).

Texas: Trinity County: Robert G. Howells (2 dry shells), Lake Livingston, Aug. 1996. Robert G. Howells (1 dry shell), Lake Livingston, 30 July 1996.

White River Drainage: Arkansas: Lawrence County: MCZ (3 dry shells), Black River at Black Rock.

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A new species of *Belocaulus* (Gastropoda: Veronicellidae) from southern and southeastern Brazil

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ABSTRACT

A new species is proposed based on material collected in the states of Minas Gerais, São Paulo, Santa Catarina, and Rio Grande do Sul, Brazil. The morphology, radula, and jaw are described and illustrated. The new species is compared to *Belocaulus angustipes*, the only species of the genus currently considered valid. The main differences are found in the penis. The new species has small projections, similar to tubercles, on the anterior region of the glans, which can be scattered or arranged in two, three or more longitudinal rows. The terminal extremity of the glans has digitiform margin. The penis base is short and poorly defined. The accessory gland is completely immersed in the tegument. The description of the new species extends the distribution range of *Belocaulus* for the states of Minas Gerais and São Paulo, Brazil.

Additional keywords: Veronicellidae, *Belocaulus*, morphology, land slug

INTRODUCTION

Veronieellidae includes terrestrial slugs of the subelass Gymnophila with pantropical distribution, with no shell, and no developed pulmonary eavity such as that observed in other terrestrial slugs of the subelass Pulmonata. Some genera have been better studied beeause they include intermediate host species for the nematodes *Angiostrongylus eostaricensis* Moreira and Céspedes, 1971, and *Angiostrongylus cantonensis* (Chen, 1935), parasites responsible for abdominal angiostrongiliasis and eosinophilic meningoencephalitis, respectively. Among the veronicellid species cited as intermediate hosts for *A. eostarieensis* are *Sarasinula plebeia* (Fisher, 1868) in Central and South America, *Phyllocaulis variegatus* (Semper, 1885), *Phylloeaulis soleiformis* (d'Orbigny, 1835), *Sarasinula linguaeformis* (Semper, 1885), and *Beloeaulus angustipes* (Heynemann, 1885) in southern Brazil, and for *A. cantonensis*, *Sarasinula marginata* (Semper, 1885) in the state of Espírito Santo, Brazil (Graeff-Teixeira et al., 1989; Graeff-Teixeira et al., 1994; Rambo, 1997; Laitano et al., 2001; Caldeira et al., 2007). Veronicellid speeies have also been eited as damaging to agricultural crops (Pereira and Gonçalves, 1949; Araújo, 1952; Santos, 1959; Thomé, 1993; Milanez and Chiaradia, 1999; Chiaradia et al., 2004; Robinson and Hollingsworth, 2004).

When Hoffmann (1925) proposed *Belocaulus*, he ineluded in the genus South American species that presented the following charaeters: penis shaped as an asymmetrical arrow or irregularly widened, vas deferens opening terminally or subterminally, and presenee of a small accessory gland behind the duct of the bursa eopulatrix and rectum, partially covered by the tegument. That author included six speeies in the genus without, however, designating a type species: *B. langsdorfi* (Férussae, 1822), *B. boetzkesi* (Miller, 1879), *B. pterocaulis* (Simroth, 1913), *B. festae* (Colosi, 1921), *B. pulcher* (Colosi, 1921) and *B. sloanei* (Cuvier, 1817). Baker (1925) designated *Vaginulus angustipes* as the type speeies of *Belocaulus* and regarded the latter as a synonym of *Angustipes* Colosi, 1922.

The synonymy proposed by Baker (1925) was not accepted by Thomé (1975), who revised the neotropical species of Veronicellidae. Aecording to Thomé (1975), *Beloeaulus* is valid and characterized by the presenee of a small aecessory gland between the rectum and the female genital pore (which, according to Thomé, is absent in *Angustipes*). He mentioned additional charaeteristics of *Belocaulus*: the reetum penetrates elose to the female genital pore, the penial gland has uniform and sinuous tubules at the base (where they are enveloped as a whole by a membrane), and the bursa copulatrix has a kidney or oval shape, with a short duct and other conneeting duet that penetrates at the base of the gland accessory.

The six species ineluded in *Beloeaulus* by Hoffmann (1925) were transferred to other Neotropieal genera by Thomé (1975): *Novovaginula* Thiele, 1931, *Simrothula* Thomé, 1975, *Colosius* Thomé, 1975, and *Veronieella* Blainville, 1817. Aeeording to Thomé (1975), *Beloeau-*

lus included two valid species: *B. angustipes*, described originally from Taquara (State of Rio Grande do Sul), and *B. aberrans* (Heynemann, 1885), described originally from Santa Cruz do Sul (State of Rio Grande do Sul). However, Pitoni and Thomé (1981) and more recently Thomé (1993) regarded *B. aberrans* as a synonym of *B. angustipes*, rendering *Belocaulus* monotypic.

Belocaulus angustipes merits special attention because of its wide distribution in southern South America (east of the Andes). It is recorded from Rio Grande do Sul and Santa Catarina, in Brazil, as well as from Uruguay, Argentina, and Paraguay (Pitoni and Thomé, 1981; Thomé, 1993; Thomé et al., 1999; Simone, 2006; Thomé et al., 2006) (Figure 1). *Belocaulus angustipes* is also recorded from Honduras and south of the United States where it is considered an introduced species (Thomé, 1989; Caballero et al., 1991; Thomé, 1993); the species distribution is discontinuous.

The study of a large number of specimens of *Belocaulus* from twelve localities revealed the existence of a new species, which distributed throughout southern and southeastern Brazil. Its morphology is very similar to that of *B. angustipes*, although both species can be distinguished from each other by some characters of the male reproductive system. The morphology, radula, and jaw of the new species are described and illustrated. It is also compared to *B. angustipes*, and the main differences and similarities are pointed out. New records of *Belocaulus* are provided in the states of Minas Gerais and São Paulo.

MATERIALS AND METHODS

The description of the new species proposed herein is based on the examination of 92 specimens from 35 lots collected from twelve different localities in southern Brazil. The material is deposited in the collections of the Museu de Zoologia, Universidade de São Paulo (MZUSP) (lots 87747, holotype, 87748–87750, paratypes), Museu de Ciências e Tecnologia, Universidade Católica do Rio Grande do Sul (MCP) (lots 7971, 7972), and in the Malacology collection of the Superintendência de Controle de Endemias, São Paulo, (SUCEN) (lots 8968, 8982–84, 8987–8995, 8997, 9005, 9006, 9016, 9019, 9020, 9031–9037, 9039, 9042, and 9043). Specimens of *B. angustipes* from Caxias do Sul, Pinhal, and Santa Maria, Brazil, and Santa Fé and Tucumán, Argentina were also examined for comparison. These are deposited at SUCEN (lots 9021, 9022, 9029, 9030 and 9038). Most of the material is preserved in 70% ethanol, although some of the material from São Paulo was fixed in Raillet–Henry. Animals were killed by submersion in filtered water, and kept in hermetically-closed recipients in the refrigerator for 48–72 hours. Prior to fixation, specimens were photographed using a Canon Digital Power Shot SD630 and observed alive. Preserved specimens were dissected under a stereomicroscope. Anatomical illustrations were made using a camera lucida. Pictures of the internal structures were obtained with a DFC 280 digital camera attached to the stereomicroscope. Digital images were merged using Automontage Pro (Synchroscopy) and Zeiss LSM Browser. Five radulae and five jaws of the new species were extracted and examined under a scanning electron microscope LEO 440 at the Museu de Zoologia, Universidade de São Paulo (MZUSP) (lots 8997, 9005, and 8983). The terminology and anatomical characters described and illustrated are in accordance to those considered diagnostic for Veronicellidae according to Thomé et al. (2006), Gomes et al. (2006) and Gomes (2007).

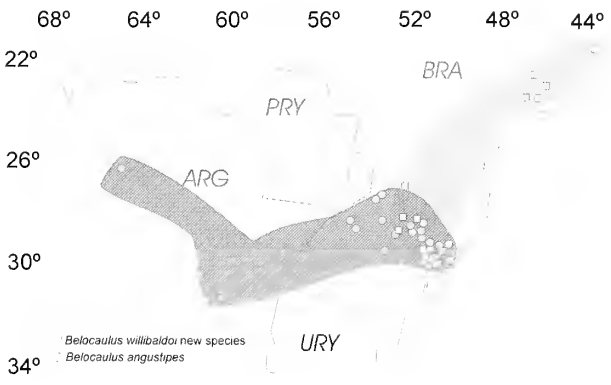


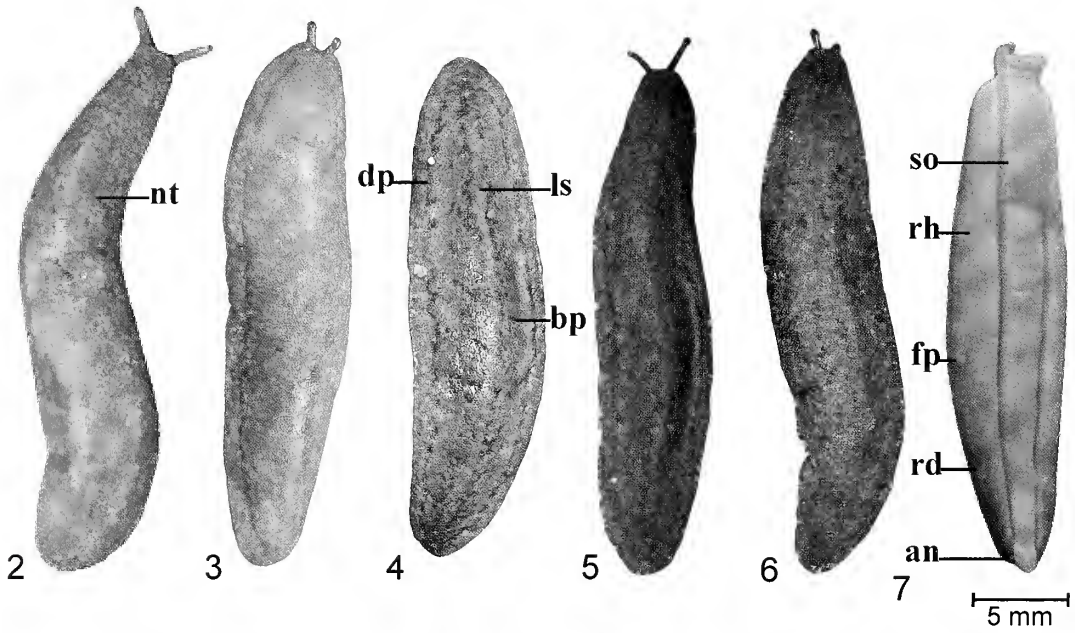
Figure 1. Map showing the distribution of *Belocaulus willibaldoi* new species and *Belocaulus angustipes*, considering literature records and lots recently collected of the latter species (from Caxias do Sul and Pinhal).

RESULTS

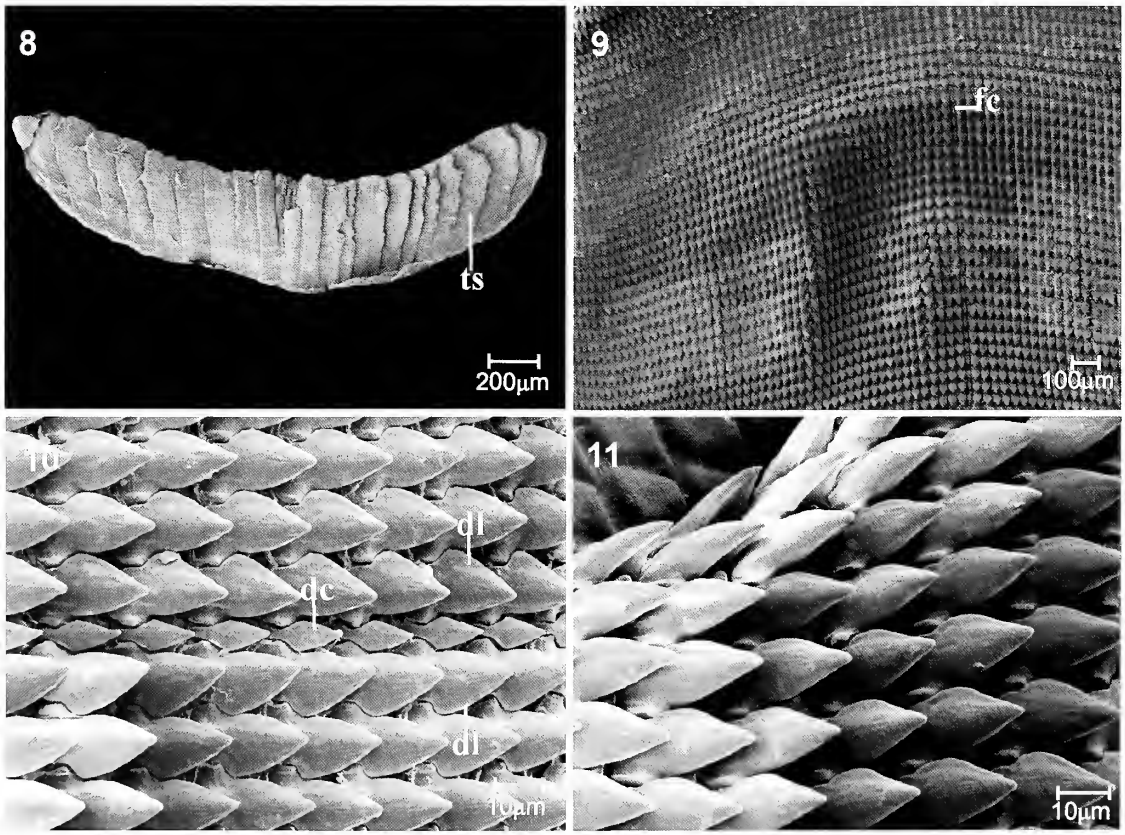
Belocaulus willibaldoi new species

Diagnosis: *Belocaulus willibaldoi* bears small projections similar to tubercles in the anterior region of the glans. These can be uneven or arranged in two, three, or more longitudinal rows. The glans presents a widened basal region, narrowing towards the extremity and ending in a digitiform margin. The penis base is short and poorly defined. The accessory gland is completely immersed in the tegument.

External Morphology: The length of the examined specimens ranges from 2.19 to 7.1 cm, the total width from 0.63 to 1.80 cm, the sole width from 0.16 to 0.52 cm, the left hyponotum width from 0.28 to 0.90 cm, and the right hyponotum width from 0.32 to 0.90 cm. Notum coloration varies from brown to light or grayish-brown (Figures 2–7). Mostly dark, slightly or strongly conspicuous black puncta are noticeable, usually scattered. In the majority of the specimens, there is a lighter, median, longitudinal stripe on the notum. In specimens with a darker notum, coloration pattern tends to be more uniform with slightly conspicuous puncta and lighter median, longitudinal stripe. The hyponotum and the sole are beige. The external borders of the hyponotum



Figures 2–7. External view of different forms of *Belocaulus willibaldoi* new speeies. **2–6.** Dorsal view showing the variation patterns of eoloration. **7.** Ventral view. Abbreviations: **an:** anus position; **bp:** blaek puneta; **dp:** dotted line delimitating the perinotum; **fp:** female genital pore; **ls:** lighter, median, longitudinal stripe; **nt:** notum; **rd:** region of darker pigmentation; **rh:** right hyponotum; **so:** sole.



Figures 8–11. Radula and jaw of *Belocaulus willibaldoi* new species. **8.** Entire jaw (Lot 8997). **9.** Middle part of the radula (lot 8997). **10.** Central part of the radula, showing lateral and eentral teeth (lot 8983). **11.** Lateral teeth fit (lot 8983). Abbreviations: **dc:** central teeth; **dl:** lateral teeth; **fc:** rows of central teeth; **ts:** transversal stripes.

can present a narrow stripe of darker pigmentation. There is a dotted line delimitating the perinotum in the majority of the specimens. The sole is narrow and surpasses the posterior limit of the body when the animal is moving. The width of the sole is always less than the width of the right hyponotum, but never equals less than half its width. The female genital pore is located ventrally, in the posterior half of the right hyponotum, while the male genital pore is located in the anterior region, under the inferior right tentacle.

Internal Morphology (Figures 8–23): Salivary glands formed by large and well differentiated acini. Anterior intestinal loop located behind the digestive gland anterior lobe. One pair of pallial and one of pedal nerves run both parallel and together to each other from the central nervous system toward the posterior portion of the body cavity. They are united (all four) throughout the entire part of the path on the sole and are slightly separated at the posterior region. The pedal aortic artery runs between the pairs of pallial and pedal nerves. It arises from a bifurcation of the anterior aortic artery near the pericardium and runs between the nerves until they terminate at region posterior of the body. The pedal gland is long, flattened, with a conspicuous, median, longitudinal, lighter stripe. The posterior extremity of the gland is free in the body cavity and receives very thin and short ducts in its extremity. The rectum penetrates in the tegument at the height of the female genital pore, behind the oviduct (Figure 12). The anus opens in the posterior region of the body, where the free end of the sole of the foot protects it. An opercular membrane protects the anal opening. The bursa copulatrix is spherical to oval-shaped and presents a short and thickened duct that opens into an atrium, into female genital pore. The canalis junctor penetrates in the bursa itself (not in the bursa copulatrix duct) (Figure 12). At the junction between the bursa copulatrix and the oviduct is a small, yellowish accessory gland completely immersed in the tegument (Figure 12). In some specimens, the accessory gland can be seen by transparency through the tegument. The penial gland presents a short and conical or long papilla with a terminal mammila and 18–26 tubules (Figures 19–22). The tubules located at the base of the papilla in the penial gland are sinuous, not distinguished by size. Some present the extremity or the median region bifurcated. From the posterior region of the penial gland extends the retractor muscle, which is connected to the penis retractor muscle and together these are inserted in the tegument. The penis is robust, with no spathe, with a small base and glans with a wide basal region narrowing toward the apical extremity (asymmetrical arrow shaped penis) (Figures 13–15). The distal extremity of the glans presents a digitiform margin (Figures 16–17). On each side of the basal region of the glans there is a whitish nervure. On the anterior region, the glans presents small projections shaped as minuscule tubercles (Figures 16–18). These are arranged in two, three or more longitudinal rows or are unevenly distributed on the anterior region

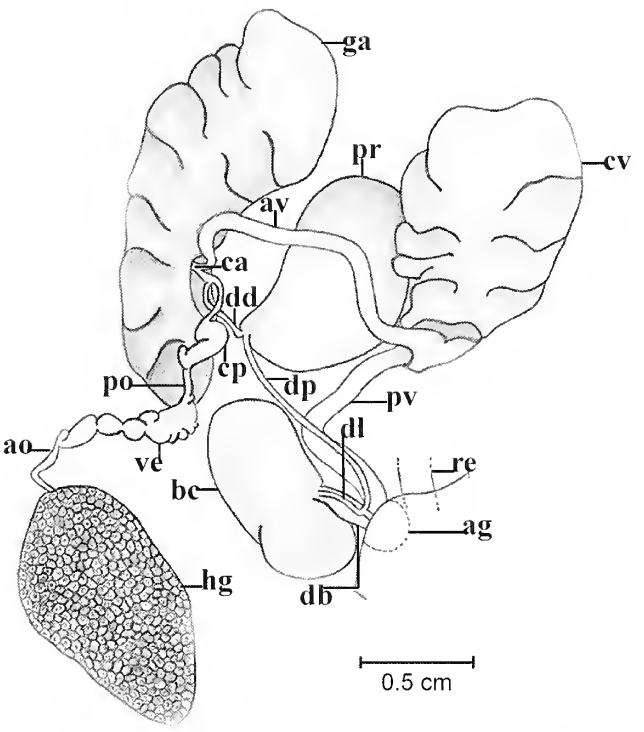
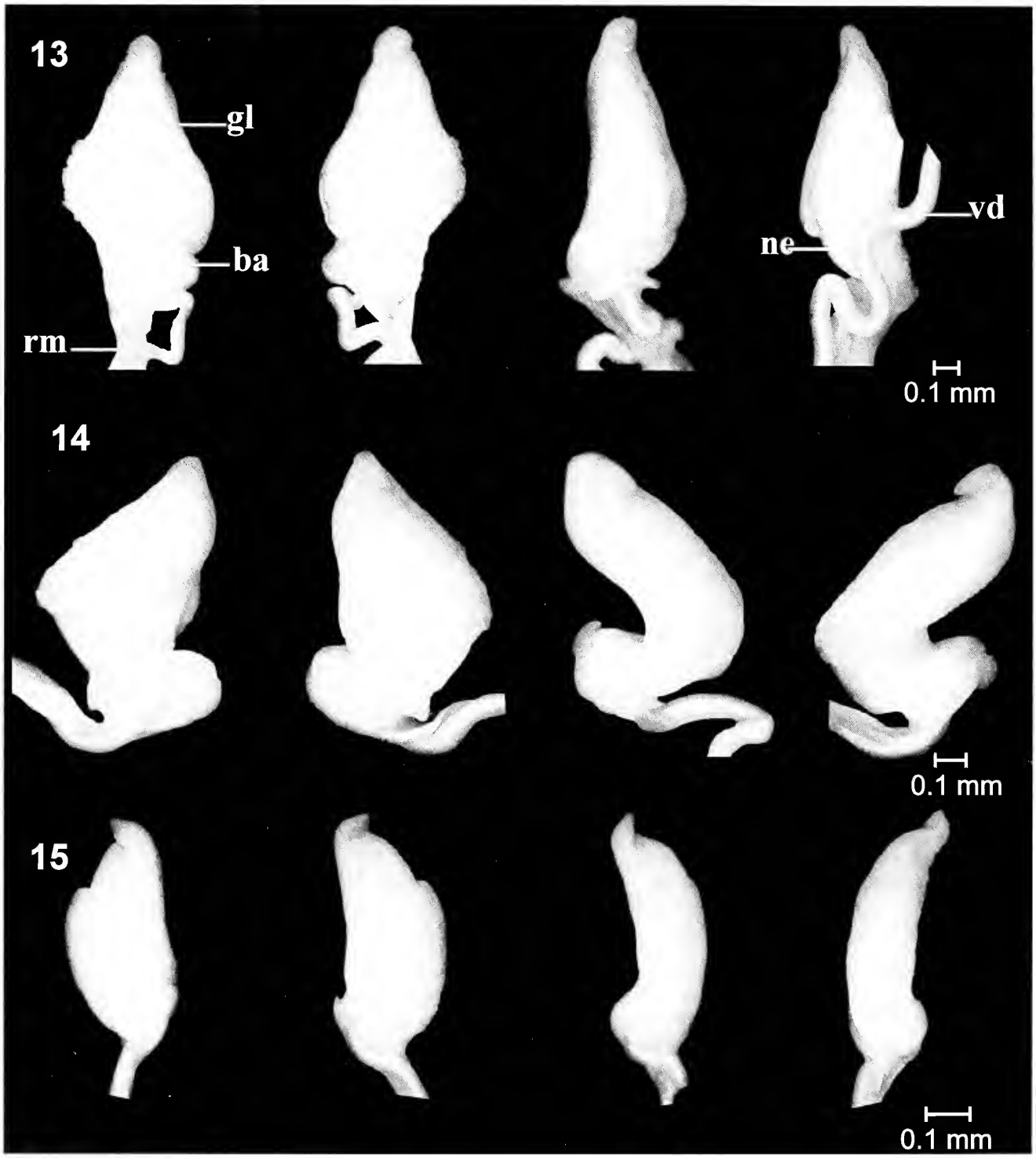


Figure 12. Part of the reproductive system of *Belocaulus willibaldoi* new species (lot 8997). Abbreviations: **ag:** accessory gland; **ao:** portion of spermoviduct; **av:** anterior region of oviduct; **bc:** bursa copulatrix; **ca:** "carrefour"; **cp:** fertilization pouch; **cv:** coiled region of oviduct; **db:** bursa copulatrix duct; **dd:** posterior distal vas deferens; **dl:** canalis junctor; **dp:** posterior proximal vas deferens; **ga:** albumin gland; **hg:** hermafrodict gland; **po:** portion of spermoviduct; **pr:** prostate; **pv:** posterior region of oviduct; **re:** part of rectum; **ve:** seminal vesicle.

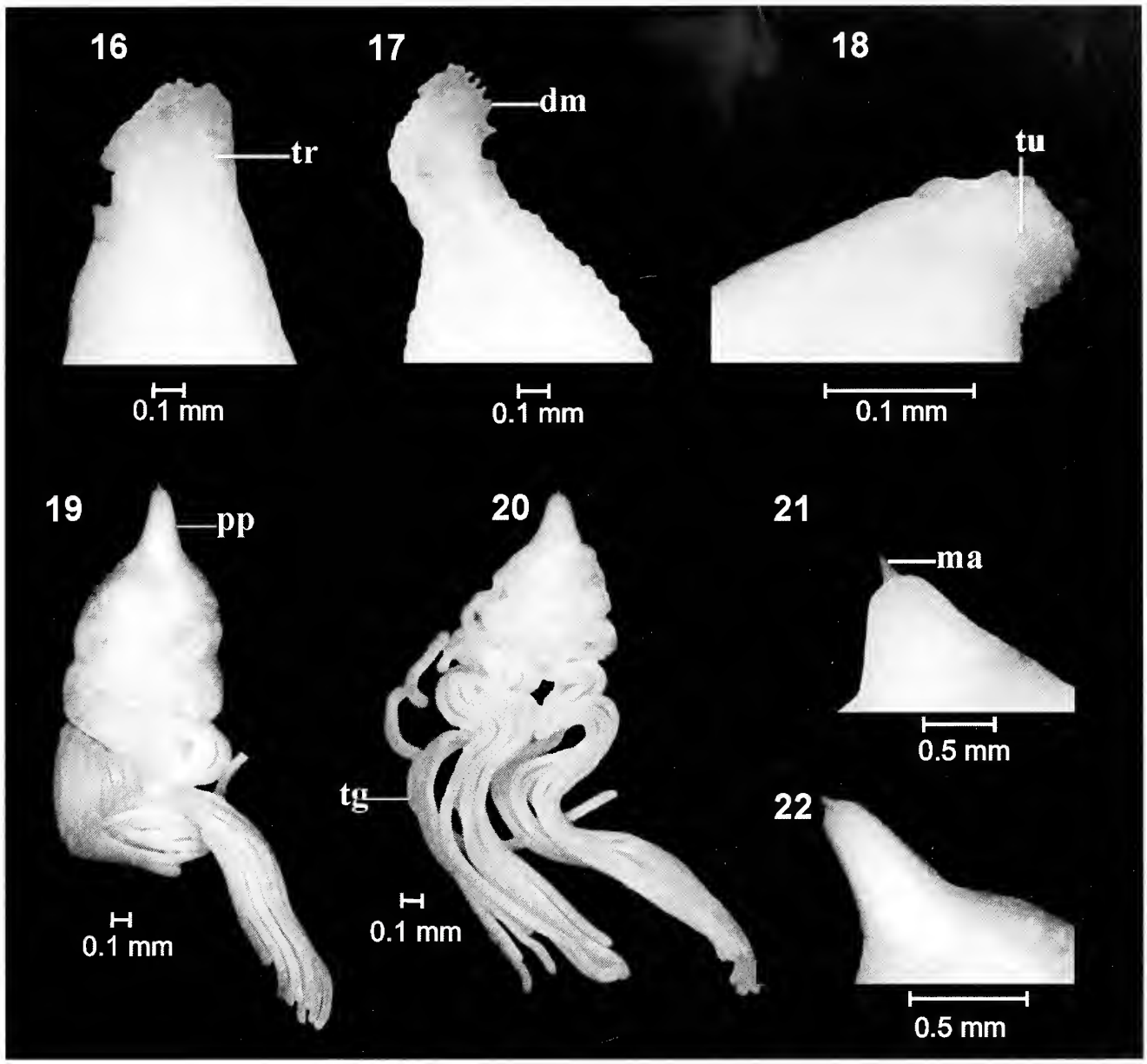
of the glans. In young animals, the digitiform margin of the opening may be inconspicuous or not developed. In young specimens the tubercles may also be absent, difficult to see, or even appear as small depressions (future tubercles). The penis and the penial gland are independently surrounded in their own muscular sheath. Both sheaths fuse to form an anterior atrium near the male genital pore (located at the base of the inferior right tentacle) (Figure 23).

The brown jaw (Figure 8) is located at the dorsoanterior region of the buccal bulb. It forms an arch composed of 21–25 transversal plates, partially covered and parallel to each other, which resemble lathes with a keel-shaped dorsal region; plates are ornamented with strong transversal and weaker longitudinal stripes. The radula (Figures 9–11) is composed of lateral teeth on each side of a central tooth. The radular formula varies from C/1+L52–55/2. The central teeth are small, triangular, and unicuspid. The lateral teeth, larger than the central teeth, are triangular and unicuspid. On the dorsal region of the apical extremity of the lateral teeth the cuspid stands out from the rest of the teeth. The lateral teeth are triangular, but the cuspid is not as prominent as in the teeth closer to the central teeth.



Figures 13–15. Four different views of the penis from three adult specimens of *Belocaulus willibaldoi* new species. **13.** Lot 8997. **14.** Lot 8995. **15.** Lot 9006. Abbreviations: **ba:** penis base; **gl:** glans; **ne:** whitish nervure; **rm:** penis retractor muscle; **vd:** anterior vas deferens.

Measurements (mm): Holotype: 7.1 cm of total length, 1.8 cm of total width, 0.5 cm of width of the sole, 0.9 cm of width of the left hyponotum and 0.9 of width of the right hyponotum. Paratypes (four specimens): total length from 4.8 cm to 6.3 cm, total width from 1.1 cm to 1.8 cm, width of the sole from 0.3 cm to 0.4 cm, width of the left hyponotum from 0.5 cm to 0.7 cm, and width of the right hyponotum from 0.6 to 0.7 cm.



Figures 16–22. Penis and penial gland of *Belocaulus willibaldoi* new species. **16–17.** Distal extremity of the glans (lot 8997). **18.** Distal extremity of the glans (lot 8995). **19.** Penial gland (lot 8995). **20.** Penial gland (lot 8987). **21.** Papilla of the penial gland (lot 8997). **22.** Papilla of the penial gland (lot 8987). Abbreviations: **dm:** digitiform margin; **ma:** mamilla; **pp:** papilla of penial gland; **tg:** penial gland tubules; **tr:** tubercle row; **tu:** unevenly arranged tubercles.

Type Material: Holotype: MZUSP 87747; four paratypes: MZUSP 87748 (one specimen), MZUSP 87749 (one specimen), MZUSP 87750 (two specimens).

Type Locality: Brazil, São Paulo State, São Paulo, Bairro Parque Fernanda I., 23° 40′ 05.89″ S, 49° 47′ 26.66″ W.

Etymology: The specific name honors Dr. José Willibaldo Thomé for his great contribution to the knowledge of veronicellids and other terrestrial gastropods.

Distribution (Figure 1): Brazil: Minas Gerais State (MG): Rio Acima; São Paulo State (SP): Caieiras, Guarulhos, Osasco, São Paulo; Rio Grande do Sul State (RS): Ernestina, Gravataí, Igrejinha, Porto Alegre, Riozinho, Sapiranga, and Vila Maria; Santa Catarina State (SC): Chapecó.

Habitat and Habit: The specimens of *Belocaulus willibaldoi* collected in MG, SP, SC, and RS were found in urban centers and surrounding areas, in gardens and soil, under tree trunks, wood, plastic and other objects

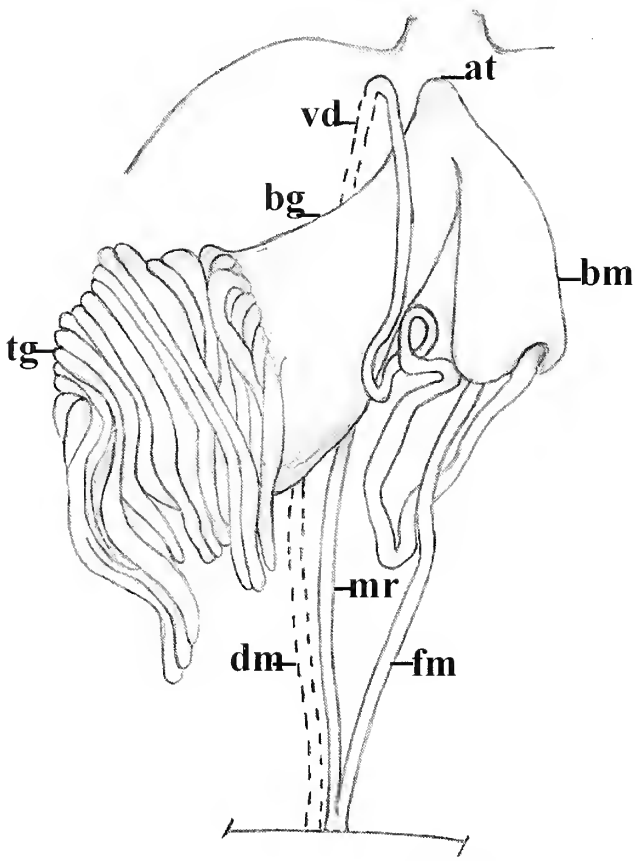


Figure 23. Penial complex of *Belocaulus willibaldoi* new species (lot 8997). Abbreviations: **at:** common atria; **bg:** muscular sheath of the penial gland; **bm:** muscular sheath of the penis; **dm:** middle vas deferens; **fm:** penis retractor muscle; **mr:** penial gland retractor muscle; **tg:** penial gland tubules; **vd:** anterior vas deferens. Size of complex: 1.8 cm.

on the ground. They are active mainly at night or, after rainy periods, during the day.

DISCUSSION

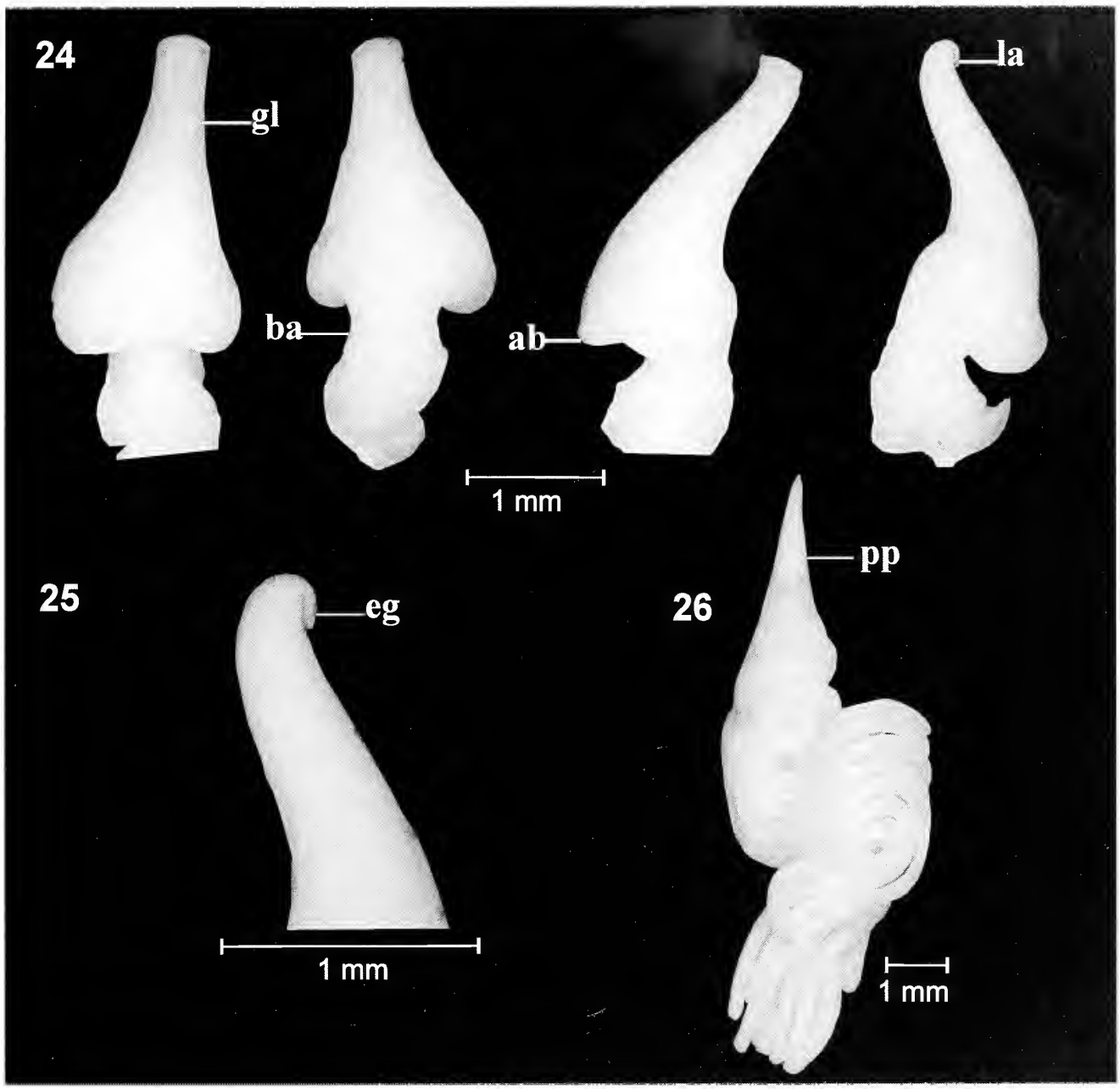
The new species described here from southern and southeastern Brazil is typical of the genus *Belocaulus* because it presents a penis shaped as an asymmetric arrow and an accessory gland connected to the female genital atrium, which are the two main characters of the genus according to Hoffmann (1925) and Thomé (1975). *Sarasinula* also includes species with an arrow-shaped penis (Gomes 2007), but penises in *Sarasinula* tend to be more symmetrical and there is no accessory gland. In addition, *Sarasinula* species are larger species and they have a more oval shape, with a different pattern of external pigmentation. The new species has relatively small size and is represented by slender slugs when compared to other species found in southern and southeastern Brazil. As *Belocaulus angustipes*, *B. willibaldoi* is a small species when compared to those of other Neotropical genera. It is slender with a strongly narrow sole. Externally, both species cannot be distinguished from each other: their coloration ranges from brown to beige or gray, in different degrees of intensity. In *B. willibaldoi* the width of the sole of the foot is smaller than the width of the right hyponotum, as described by Santos and Thomé (1999) for *B. angustipes*.

Internally, both species are also very similar. There are no differences regarding the digestive, circulatory, and nervous systems. The main differences are observed in the male reproductive system, where the main diagnostic features in Veronicellidae are found (Semper, 1885). Small variations are also observed in the accessory gland, radula, and jaw. The penis of *B. willibaldoi* is robust, with no spathe, with a small base and a glans, with a wide base narrowing towards the apical extremity with a digitiform margin. The anterior region of the penis bears minuscule tubercles, which can be scattered or arranged in two, three, or more longitudinal rows. The new species is distinguished from *Belocaulus angustipes* which, according to Pitoni and Thomé (1981) and Santos and Thomé (1999), presents only a short, screw-shaped socket, distal extremity widened and truncated, and glans with rhomboid extremity. In some specimens the glans can be bilobed. In *B. angustipes*, adjacent to the penis base, the glans is projected backwards over itself (in one side of the penis) (Figures 24–26). In *B. willibaldoi* the penis base is shorter and less defined than in *B. angustipes*. In both species a labium is frequently formed on the glans extremity, which folds back covering the opening of the vas deferens.

The penial gland, in general, is similar in both species, differing only in the number of tubules. In *B. willibaldoi*, the penial gland presents from 18 to 26 tubules, while in a *B. angustipes* it presents 13–22 tubules (Pitoni and Thomé 1981; Santos and Thomé, 1999). A terminal mammila was observed in the papilla extremity in *B. willibaldoi*. Even though Pitoni and Thomé (1981) and Santos and Thomé (1999) did not mention the existence of a mammila in *B. angustipes*, it was observed in all specimens examined in this study. This mammila is not, however, so conspicuous due to the fact that the papilla is narrower when compared to that of *B. willibaldoi*.

The accessory gland of *B. willibaldoi* is completely inserted in the tegument, differing from that of *B. angustipes* in which, according to Thomé (1975) and Silva and Thomé (1995), it can be totally or partially covered by the tegument. According to Silva and Thomé (1995), the accessory gland releases a lubricant secretion, probably used during copulation, toward the female genital pore, since it opens in this region.

The morphology of the jaw of *B. willibaldoi* is similar to that described for *B. angustipes* by Thomé and Chaves (1997). The jaw and the radula of both species are distinguished only by the number of jaw plates and the number of teeth per row. According to those authors, the jaw of *B. angustipes* includes 19–22 plates, while *B. willibaldoi* includes 21–25 plates. Based on the characters presented by Thomé and Chaves (1997) for



Figures 24–26. Penis and penial gland of *Belocaulis angustipes* (lot 9030). **24.** Four different views of penis. **25.** Distal extremity of the glans. **26.** Papilla of penial gland; **ab:** glans reflected backward over itself; **ba:** penis base; **eg:** distal extremity of glans, without tubercules; **gl:** glans; **la:** labium; **pp:** papilla of penial gland without a mamilla.

the radula of *B. angustipes*, we notice that the radular formula in *B. willibaldoi* (C/1+L52–55/2) is higher, since *B. angustipes* presents C/1+L34–38/2.

Belocaulus, which was regarded by Pitoni and Thomé (1981) as a monotypic genus, includes another species, *B. willibaldoi*, which occurrs in the states of Minas Gerais, São Paulo, Santa Catarina, and Rio Grande do Sul. The known records of *B. angustipes* reaeh the most southern point within the distribution of the genus, including localities in Argentina and in Brazil (states of Rio Grande do Sul and Santa Catarina). Although both species have been recorded from Rio Grande do Sul and Santa Catarina, it is probable that *B. angustipes* occurs more to the south, while *B. willibaldoi* more to the north. With the description of *B. willibaldoi*, the distribution range of *Belocaulus* is extended to the states of Minas Gerais and São Paulo, Brazil.

ACKNOWLEDGMENTS

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Sensory structures on the siphons of wood-boring bivalves (Pholadidae: Xylophagainae: *Xylophaga*)

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ABSTRACT

Deep-sea bivalves of Xylophagainae spend their entire post-metamorphic lives boring into wood that has fallen to the seafloor. Although their boreholes seemingly provide a protected, imperturbable habitat, scanning electron microscopy reveals that the siphons of three species of *Xylophaga* examined carry elaborate structures that are interpreted as chemoreceptors or mechanoreceptors. Sensory structures occur on the siphonal surface of *Xylophaga oregona* Voight, 2007, and *X. multichela* Voight, 2008. The large complex papillae of *X. multichela* are scattered on the distal incurrent siphon and arrayed in two longitudinal rows along its dorsal surface. The distal incurrent siphon of *X. oregona* carries minute structures, barely projecting above the surface, that are crowned by tufts of cilia. Both siphonal openings of *X. microchira* Voight, 2007, carry cirri. At the excurrent opening, cirri have long cilia emerging from terminal pits. At the incurrent opening, cirri form two rings. The inner cirri appear to be unique in that cilia emerge from between scales that cover their inner surfaces. The structures observed may be useful in species taxonomy and systematics, but we suspect that their elaboration is linked to predation pressure, which might relate to depth distribution.

Additional keywords: Goblet organs, scanning electron microscopy, depth distribution, predation, deep-sea

INTRODUCTION

Deep-sea bivalves of the Xylophagainae spend their post-metamorphic lives using toothed ridges on their shells to bore into wood that has fallen to the seafloor. Only the siphons emerge from the resulting dead-end boreholes. Although most bivalves suspension-feed by extracting food from water moving across the gills, the small ctenidia and the labial palps of representatives of Xylophagainae lack significant sorting mechanisms (Purchon, 1941). Purchon (1941) proposed that these animals ingest wood scrapings, which are digested with the help of endosymbiotic bacteria (Distel and Roberts, 1997).

This paper reports scanning electron microscope (SEM) investigations of the siphons of three species of *Xylophaga* Turton, 1822, the most diverse genus of wood-boring bivalves, with more than 50 named species (Voight, 2008). Sensory structures, known from the siphons of a few shallow-water bivalves representing a wide taxonomic range (e.g., Hodgson and Fielden, 1984; Pekkarinen, 1986; Fishelson, 2000), are here documented in three congeneric species. Differences among the structures in these species are largely consistent with inferred ecological differences.

MATERIALS AND METHODS

Although most Xylophagainae species are known only from their type localities, recovery of experimental wood deployments from the deep Northeast Pacific (Voight, 2007) provided abundant specimens of the Xylophagainae and allowed for SEM study of the siphons of *Xylophaga oregona* Voight, 2007 (Field Museum of Natural History, Chicago, FMNH 308705) and of *X. microchira* Voight, 2007 (FMNH 309602), from 2211 m depth. Specimens were recovered inside a lidded box on a subsea vehicle in 2003 and 2004, respectively, fixed in 8% buffered formalin in seawater, and transferred within 48 hours to 70% ethanol. No attempt was made to relax the specimens prior to fixation. A single lot of *X. multichela* Voight, 2008 (Scripps Institution of Oceanography Benthic Invertebrate Collections, SIO-BIC M11567) was collected by trawl in 1973 from between 106 and 113 m depth, fixed in formalin and later moved to 80% ethanol. All specimens were dehydrated in ethanol and then critical point-dried with CO_2.

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Each sample was sputter-coated with gold palladium in a Hummer sputter-coater and examined using a Zeiss Leo Evo 60 Scanning Electron Microscope (SEM). Ecological data reported here are from species descriptions (Voight, 2007, 2008).

RESULTS

Images of these specimens are clear, despite the absence of specific preparation for SEM studies. The lack of appropriate fixation is not likely to have resulted in the different morphologies and distributions of structures seen, although it may have induced some artifacts in the fine details of the siphon surfaces. Therefore we focus on the morphology of the large structures. The species, which all have an incomplete siphon (the excurrent is distinctly shorter than the incurrent siphon), are discussed below.

Xylophaga oregona (Figures 1–4), competitive dominant, depth 1550–2211 m

For a view of the whole siphon of *Xylophaga oregona*, see Voight (2007, Figure 8A). The excurrent opening lies under an apparently featureless C-shaped hood of tissue near the posterior valve (Figure 1). The incurrent siphonal opening of *X. oregona* lacks cirri (Figure 2). The incurrent siphon distal to the excurrent opening is slightly dorsally flattened; low marginal walls border the dorsal surface (Figure 1). The surface of the incurrent siphon carries concentric ridges (Figures 1, 2). Distally, very small (12–18 µm diameter) structures (Figures 3, 4) emerge apparently at random from the surface ridges. Each structure has a terminal pit from which numerous cilia emerge (Figure 4).

Xylophaga multichela (Figures 5–8), ecology unknown, depth 106–119 m

For a full view of the siphon of *Xylophaga multichela*, see Voight (2008, Figure 1A). In *X. multichela*, the excurrent siphon opens near the posterior valve to form a U-shaped base of a longitudinal groove (Figure 5). Papillae border the groove and are scattered on the lateral and ventral distal incurrent siphon (Figure 6). The opening of the incurrent siphon lacks cirri; however, its tip is morphologically distinct with concentric ridges, rather than a smooth or papillate surface (Figure 7). The papillae bordering the groove (Figure 6) carry terminal cilia (Figure 8) and form fringed lappets. The papillae on the distal siphon also have terminal cilia and appear morphologically similar to, but smaller than, those lateral to the groove. Concentric folds (annulations) on the papillae (Figure 6) and differences in the visibility of the papillae among specimens in light microscopy (unpublished data) suggest that the cilia-topped papillae of the lappets and on the distal siphon are retractable.

Xylophaga microchira (Figures 9–15), early colonist, depth 1550–2656 m

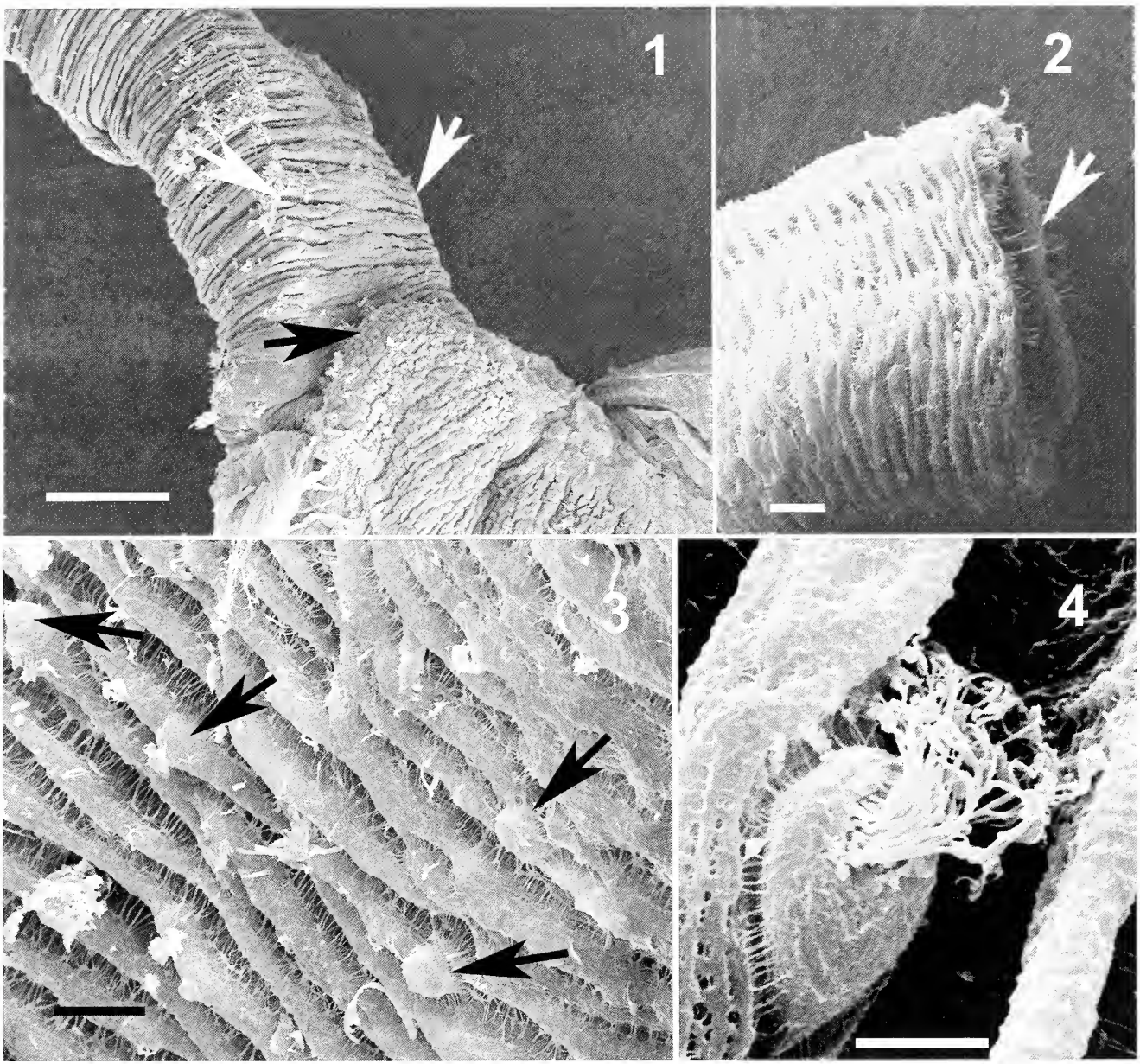
The siphon of *Xylophaga microchira* is circular in cross section and both siphonal openings carry cirri (Figure 9). The opening of the excurrent siphon is near the middle of the siphon and is flanked by very long cirri (up to 420 µm; Figure 10). Cilia emerge from pits at the tips of the cirri (Figure 11). Although the surface of the siphon is ridged, which may be due to contraction, structures such as those seen in *Xylophaga oregona* (Figure 3) appear to be absent.

The incurrent opening has two concentric rings of cirri (Figure 12). The outer cirri are smooth whereas the inner cirri, especially their inner surfaces, appear scaly (Figures 13, 14). Cilia emerge from between the scales (Figures 13, 14) and are densest at the periphery of each cirrus (Figures 14, 15).

DISCUSSION

All structures documented here, whether on the siphonal surface or at the tip of a cirrus (= tentacle sensu Fishelson, 2000), share a terminal opening with an emergent tuft of equal-length cilia. The absence of a long central flagellum leads us to interpret these structures as sensory organs, reportedly common in bivalves (Fishelson, 2000). Distinguishing between mechanoreceptor and chemoreceptor cells is difficult (Hodgson and Fielden 1984), even if neuronal connections are traced (Fishelson, 2000). Earlier comparison of transmission electron microscopy (TEM)-documented ultrastructure of sensory cells to that of known chemoreceptors or mechanoreceptors was said to identify modality of the cells (e.g. Jouin et al., 1985; Chia and Koss, 1989). However, variability in the fine structure of sensory cells led Schaefer (2000: 208) to question this method. Behavioral and physiological data are integral to assign function to sensory cells (Schaefer, 2000; Zhadan et al., 2004). Given that these representatives of *Xylophaga* live inside wood on the ocean floor, at depths of over 2 km, and no material was suitably fixed for TEM study, the modality of the sensory structures documented here cannot be assigned. In general, chemoreceptors have been considered to be the most abundant sensory structure on bivalve siphons (Fishelson, 2000), however, the goblet organs of *Macoma balthica* (Linnaeus, 1758) may be mechanoreceptors (Pekkarinen, 1984).

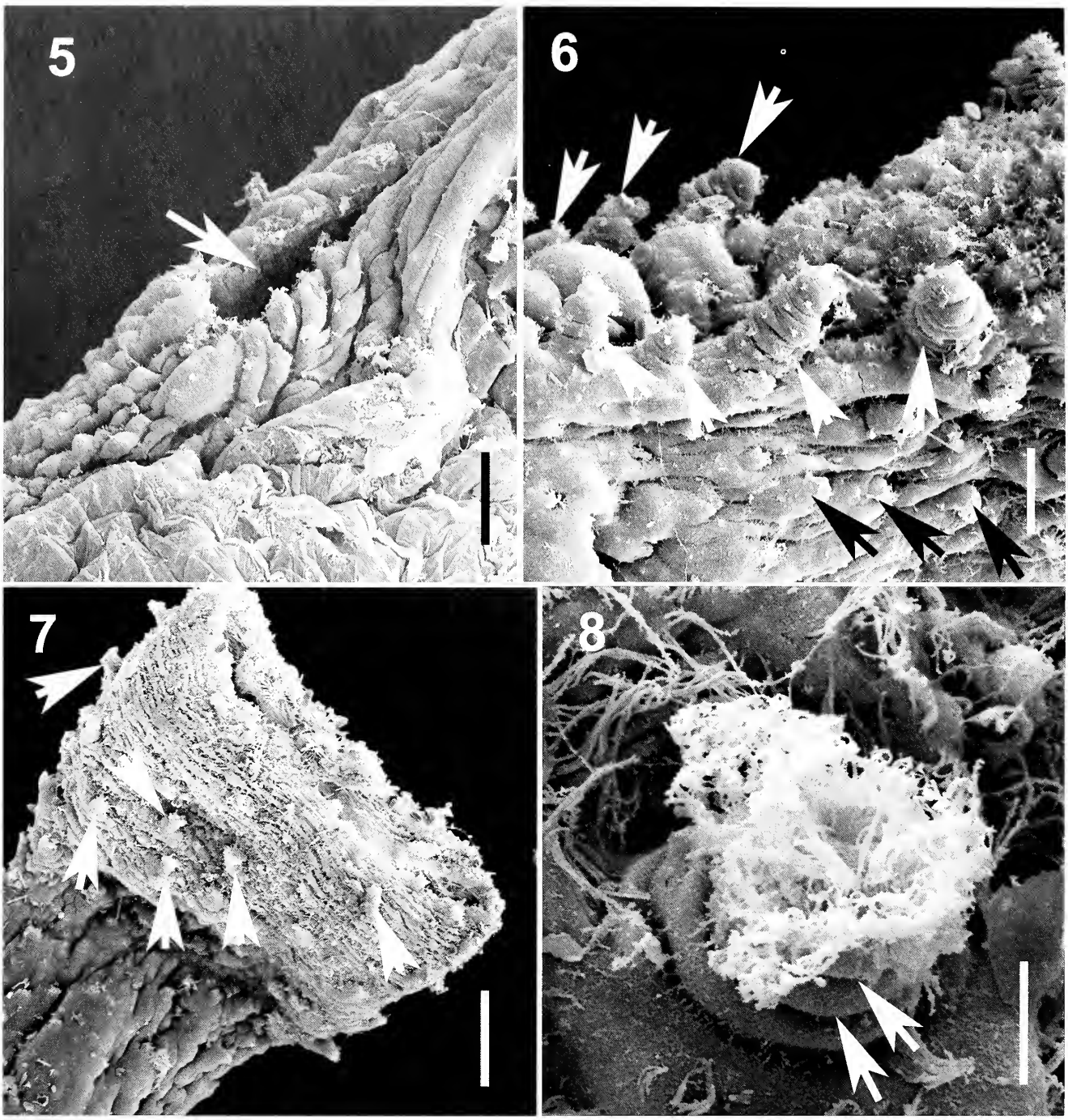
These SEM images reveal that the distribution, shape and size of the sensory structures (Figures 1–15) differ distinctly among these wood-boring bivalves. *Xylophaga oregona* (Figures 1–4) and *X. multichela* (Figures 5–8) share an excurrent siphon that is truncated near the shell (Voight, 2007, 2008); their sensory structures lie on the siphonal integument, in contrast with those on cirri at siphonal openings in *X. microchira*, a species with the excurrent opening near the middle of the siphon (Figures 9–15). These data are consistent with the hypothesis (Voight, 2007), based on differences in siphonal



Figures 1–4. *Xylophaga oregona*. **1.** Excurrent siphon. Black arrow indicates hood of tissue over the opening of the excurrent siphon. White arrows indicate marginal ridges that border a longitudinal flat area of the dorsal siphon. Scale bar = 200 µm. **2.** Tip of incurrent siphon. Arrow indicates the edge of the siphon; note the lack of cirri. Scale bar = 40 µm. **3.** Surface of distal incurrent siphon. Note the ridged appearance and the round projections indicated by arrows. Scale bar = 20 µm. **4.** Finer detail of a round structure indicated in Figure 3. Scale bar = 6 µm.

allometry and overall appearance, that the truncated excurrent siphons are not uniquely derived.

The round structures of *Xylophaga oregona* (Figures 3, 4) strongly resemble the goblet organs detailed by Pekkarinen (1984 Figures 8 and 11, 1986 Figure 5) in the veneroid bivalve *Macoma balthica*, which is only distantly related to the myoid *Xylophaga* species considered here. In both species, the small (10–20 µm) structures are associated with ridges on the distal incurrent siphonal surface, and have long cilia that emerge from a central opening (Figure 4) (Pekkarinen, 1984, 1986). The subtle shape differences could relate to differences in fixation. The goblet organs of *M. balthica* form six longitudinal rows that correspond to the course of the main longitudinal nerves (Pekkarinen, 1984, 1986); sensory structures in *X. oregona* appear to be randomly arranged. Apparent goblet organs, termed type III sensory organs by Hodgson and Fielden (1984) and Ansell et al. (1999), have also been observed on incurrent siphons of the veneroid *Donax trunculus* Linnaeus, 1758 (Fishelson, 2000, Figure 5H).



Figures 5–8. *Xylophaga multichela*. **5.** Opening of the excurrent siphon. Arrow indicates longitudinal groove originating at the opening. Scale bar = 40 µm. **6.** Two rows of papillae (white arrows) form fringed lappets lateral to groove that extends distally from the opening of the excurrent siphon. Black arrows indicate a row of papillae inferior to the lappets. Scale bar = 40 µm. **7.** Tip of incurrent siphon. Note the ridged surface at the siphonal tip and randomly scattered cirri (arrows). Scale bar = 80 µm. **8.** Finer detail of a lappet from the distal incurrent siphon. Note the tuft of equal-length cilia emerging from the center. Arrows indicate folds on a cirrus. Scale bar = 8 µm.

Bivalves living in high-energy habitats with heavy sedimentation tend to have elaborate, branched cirri on the incurrent siphon (Fishelson, 2000). Turner (1971) suggested that the elaborate cirri of shipworms (teredinids) form a sieve across the incurrent opening to protect the animal from debris. However, Lopes and Narchi (1998) found that the tentacles did little themselves to block the entrance to the incurrent siphon in the teredinid *Nausitora fusticula* (Jeffreys, 1860), rather contraction of the siphon base served to block the opening. The incurrent siphon of *Xylophaga microchira* carries structures highly compatible with a sieving function. In this species, the

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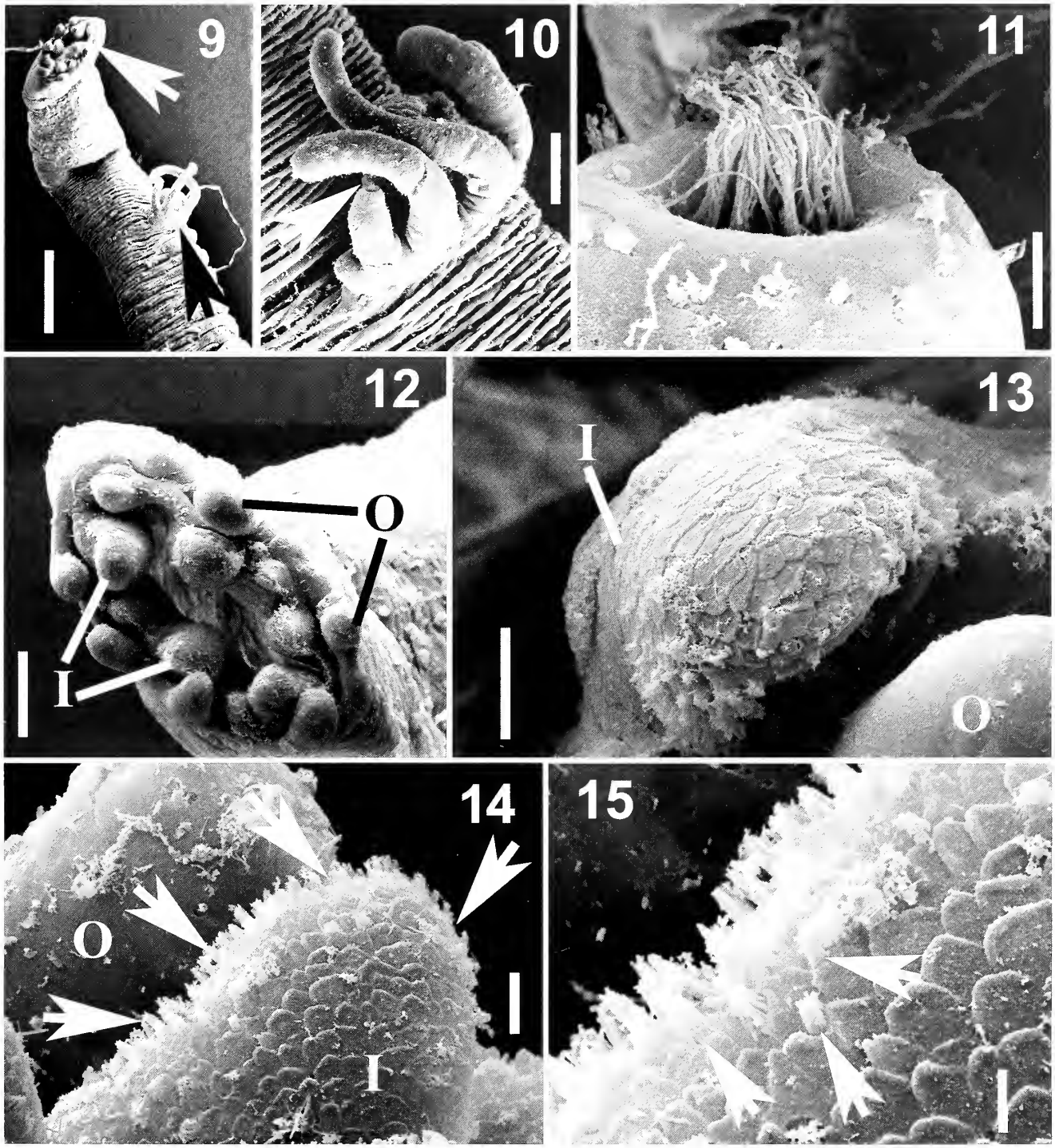

Figures 9–15. *Xylophaga microchira*. **9.** Siphon. White arrow indicates the opening of the incurrent siphon, black arrow indicates the opening of the excurrent siphon. Note ridged surface of siphon. Scale bar = 400 µm. **10.** Flap of mega-cirri at excurrent siphonal opening. Tip of cirrus indicated by the arrow. Scale bar = 80 µm. **11.** Finer detail of cirrus noted in Figure 10. Scale bar = 6 µm. **12.** Incurrent opening with two rings of cirri. I indicates inner cirri; O indicates outer cirri. Scale bar = 80. **13.** Finer detail of inner cirrus. Note that the inner surface of the inner cirrus (I) differs from that of the outer cirrus (O). Scale bar = 20 µm. **14.** Finer detail of an inner cirrus (I); outer cirrus (O). Note the scaly surface and the arch of cilia indicated by arrows. Scale bar = 8 µm. **15.** Finer detail of cilia of inner cirrus. Arrows indicate area from which cilia emerge. Scale bar = 4 µm.

inner surfaces of the inner cirri appear scaly (Figures 13–15); cilia emerge from between the scales and at their margins. The distribution of cilia is such that if these cirri were bent inwards to block the incurrent opening, penetration of the opening would perturb the maximum number of cilia, generating the maximum sensory stimulus. *Xylophaga microchira* is considered to be specialized for rapid colonization of new wood-falls and shows frequent damage on the incurrent siphon consistent with cropping by predators (Voight, 2007). Polychaetes are suggested to enter the incurrent siphon of wood-boring bivalves (Dean, 1992); this siphonal sensory system may speed the bivalve's defensive retraction of the siphon.

Excurrent siphons of most bivalves typically bear relatively smaller, simpler extensions than do the incurrent siphons (Fishelson, 2000). However, large, fairly complex sensory receptor-bearing structures occur at the excurrent opening of *Xylophaga microchira* (Figure 10, 11). In *X. multichela*, papillae on the dorsal incurrent siphon border the groove that appears to be a continuation of the excurrent siphon (Figure 6), although the papillae have been illustrated very near the excurrent opening in similar species (Turner, 2002). Near the incurrent opening of this species, smaller papillae are scattered over the surface of the incurrent siphon (Figure 7). The comparatively elaborate sensory arrays near the excurrent siphonal openings of these species remain enigmatic.

Given the broad ecological similarities of these wood-boring species and their congeneric status, differences documented here are counter-intuitive; they may reflect fine-scale ecological differences or possibly depth distribution. The species with most sensory structures, *Xylophaga multichela*, lives around 106 m depth; sensory input may help it survive in the more predator-rich continental shelf depths (Vermeij, 1987), as hypothesized by Voight (2008). Specimens of *X. oregona* have the fewest sensory structures. Boreholes of this species are lined with fecal chimneys, which, in addition to lowering oxygen tension (Voight, 2007), may minimize or confound chemical cues or muffle mechanical stimulation. In extremely high densities, however, siphons of this competitively dominant species can extend well beyond the wood, conceivably allowing chemical cues to be received (personal observation, JRV). No simple environmental variable appears to be clearly correlated with the complex sensory structures documented here.

ACKNOWLEDGMENTS

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An unusual new genus and a new species of Buccinulidae (Neogastropoda) from the Magellanic Province

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ABSTRACT

A new genus and species of the family Buccinulidae is described from the Southwestern Atlantic in Argentine waters. *Jerrybuccinum malvinense* new genus and species combines the conchological characters of Fasciolariidae with the radula of Buccinulidae.

Additional keywords: Gastropoda, southwestern Atlantic, *Jerrybuccinum malvinense*, new genus, new species

INTRODUCTION

The intensive collecting efforts of the United States Antarctic Program (USAP) in the Antarctic and Magellanic regions yielded rich collections, which stored at the National Museum of Natural History, Smithsonian Institution.

These collections have been the source of a vast number of new species of mollusks described in several papers and monographs (e.g. Dell, 1990; Harasewych and Kantor, 1999; Pastorino, 1999; Harasewych et al., 2000; Pastorino and Harasewych, 2000; Pastorino, 2002; Harasewych and Kantor, 2004; Harasewych and Pastorino, in press). There remain to be studied, however, a number of species with novel combinations of anatomical features and shell morphology. One of the new species collected off the Falkland Islands (Islas Malvinas) demonstrated the unusual combination of a fasciolariid-looking shell with a buccinulid radula.

In this paper we describe as new a species that possesses radular and conchological characters that preclude its inclusion into any presently recognized genus of Buccinoidea.

MATERIALS AND METHODS

The specimens here described are housed in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). They were collected by R/V Eltanin. The shells with dried-out bodies were re-hydratated to facilitate dissections. After cleaning with diluted bleach, air-dried, mounted on glass slides, and coated with gold-palladium radulae were studied with help of a scanning electron microscope at USNM. Most photographs were taken using a digital camera. All images were digitally processed.

SYSTEMATICS

Class Gastropoda Cuvier, 1797
Order Neogastropoda Wenz, 1938
Superfamily Buccinoidea Rafinesque, 1815
Family Buccinulidae Finlay, 1928

Genus *Jerrybuccinum* new genus

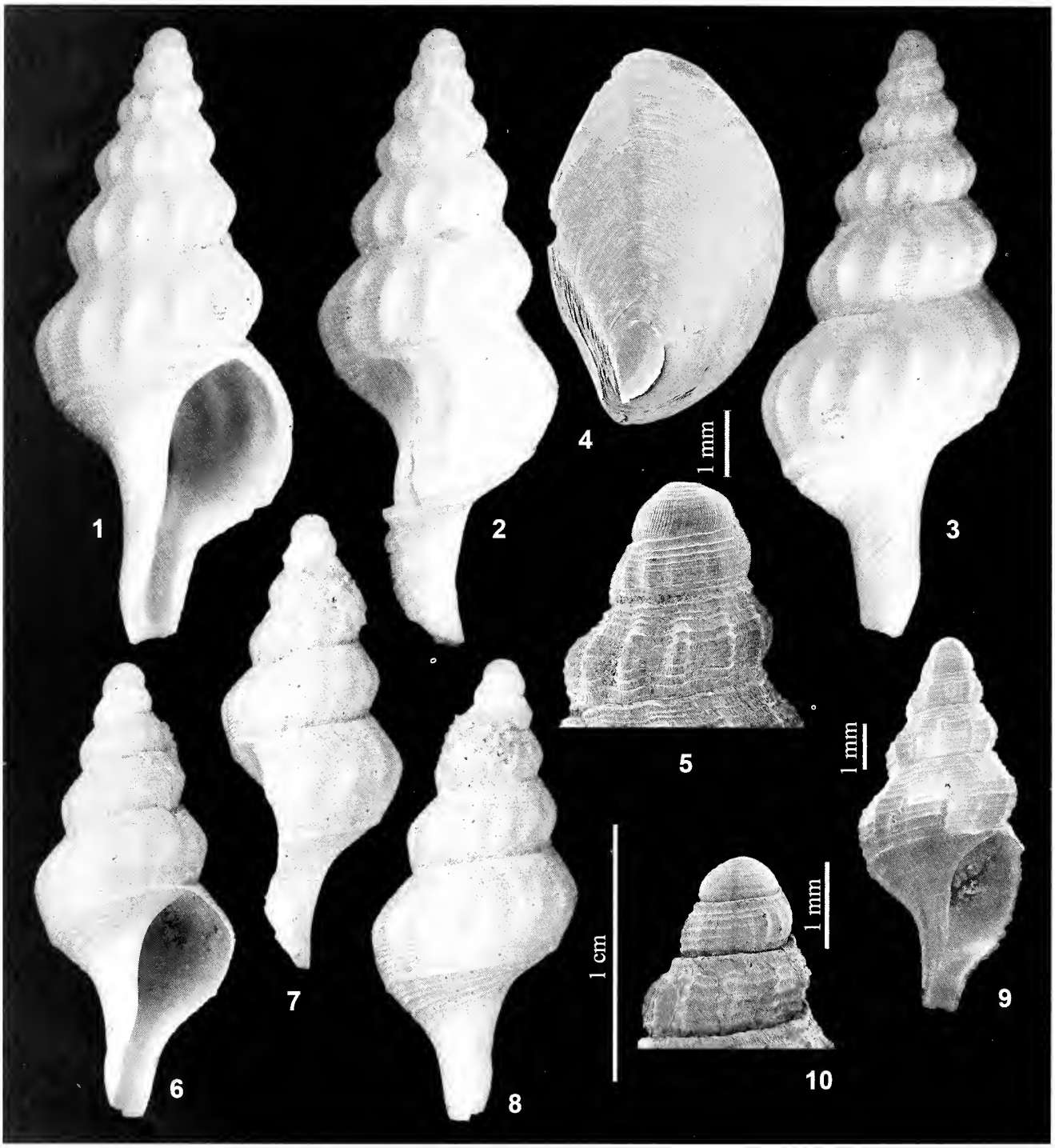
Type Species: *Jerrybuccinum malvinense* new species, by original designation. (Currently the only species included into the new genus is the type species.)

Description: Shell fusiform, with tall spire and long attenuated siphonal canal. Protoconch paucispiral, ornamented by spiral threads and closely spaced axial ribs; protoconch-teleoconch transition very weak, marked by the appearance of the axial folds. Spiral sculpture of low and narrow spiral ribs, raised, and rounded on the top keel that delimitates the shell base. Axial sculpture of growth lines and high, closely spaced axial folds. Radula triserial, with rectangular unicuspid rachidian teeth and tricuspid lateral teeth with long, stout basal plates.

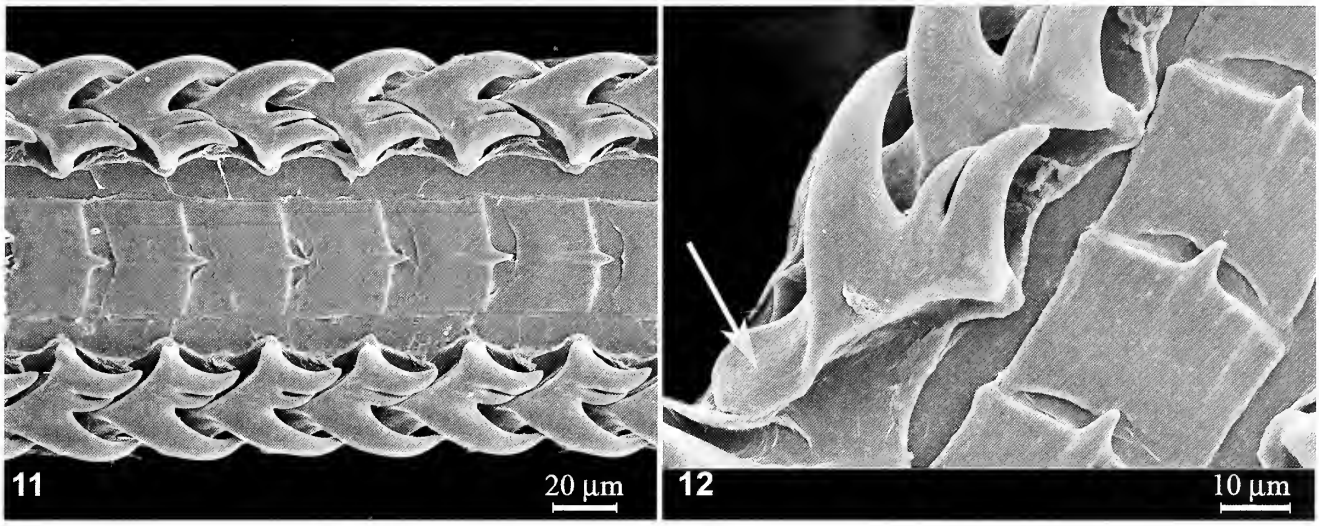
Etymology: The genus is named after our colleague and mutual friend Miroslav (Jerry) Harasewych, curator of mollusks at the National Museum of Natural History, Smithsonian Institution.

Jerrybuccinum malvinense new species
(Figures 1–12)

Description: Shell strong, fusiform with tall spire and long attenuated siphonal canal, of 2.5 protoconch and slightly over 5 teleoconch whorls. Protoconch paucispiral, evenly rounded (Figure 5), ornamented by 5 unevenly spaced spiral threads and closely spaced thin, but



Figures 1–10. *Jerrybuccinum malvinense* new species. **1–5.** Holotype, USNM 898847, Falkland Islands (Islas Malvinas), 52°00' S, 56°36' W, R/V Eltanin, cruise 7, sta. 558, 14 Mar. 1963, 646–845 m. **1–3.** Shell. **1.** Apertural view. **2.** Lateral view. **3.** Dorsal view. **4.** Operculum. **5.** Protoconch. **6–8.** Paratype, USNM 898774. Falkland Islands (Islas Malvinas), W of Beauchene Island, 53°06' S, 59°24' W, R/V Eltanin, cruise 6, sta. 340, 03.12.1962, 567–578 m. **6–8.** Shell. **6.** Apertural view. **7.** Lateral view. **8.** Dorsal view. **9–10.** USNM 887765, off Cape Horn, 56°06' S, 66°19' W, R/V Eltanin, cruise 9, sta. 740, 18 Sep. 1963, 384–494 m, shell length = 8.1 mm. **9.** Apertural view of the shell. **10.** Protoconch. Figures 1–3 and 6–8 at same scale, scale bar = 1 cm. Figures 4, 5 at same scale, scale bar = 1 mm.



Figures 11–12. Radula of *Jerrybuccinum malvinense* new species. **11.** Dorsal view of the central portion of the radular membrane. **12.** Bending plane of the membrane. The basal projection of the lateral tooth is marked by an arrow.

distinct, axial ribs. Protoconch-teleoconch transition not clear, marked by appearance of axial folds. Protoconch diameter around 2.1 mm, exposed protoconch height 1.75 mm. Teleoconch whorls strongly convex, slightly angulated at periphery, separated by shallow, slightly adpressed suture. Spiral sculpture of sharp, low, and narrow spiral ribs, separated by slightly wider interspaces. Twenty-two ribs on penultimate whorl, upper one adjoining suture, slightly wider than other ribs. Last whorl with raised keel, rounded in cross-section. Last whorl with 23 ribs above keel that delimits shell base, ribs below keel more pronounced, 28 in total on shell base and canal. Axial sculpture of raised growth lines that produce reticulated structure while crossing spiral ribs and high, closely spaced axial folds, 13 on body whorl and 13 on the penultimate whorl. Folds protrude from suture to suture on spire whorls and from suture to keel on the last whorl. Aperture wide, oval, constituting 0.34 of shell length (without siphonal canal). Outer lip evenly rounded and slightly reflected outward. Inner lip with narrow callus extending to parietal wall. Siphonal canal well defined, long, constituting about 0.17 of shell length, slightly curved to left but not crossing shell axis. Shell covered by thin, light-yellow periostracum. Shell color under periostracum uniform off-white.

Operculum ovate (Figure 4), elliptic, with subcentral nucleus, external surface covered by concentric growth lines where new growth partially overlap old ones, resulting in lamellose surface, particularly on internal margin. (Measurements as in holotype.)

Radular ribbon (Figures 11–12) long (2.98 mm, 0.36 AL), narrow (~130 µm), triserial, consisting of 90 rows, most posterior 9 rows nascent. Rachidian teeth narrow (~40 µm), with anteriorly very slightly arched rectangular basal plate and single sharp cusp. Lateral teeth with long, stout basal projection (marked by an arrow on Figure 12), attached at acute angle (~50°) to axis of radular ribbon, with 3 cusps, outer largest and central shortest situated closer to the inner cusp.

Type Material (Figures 1–8): (Measurements in Table 1) Holotype (Figures 1–5), USNM 898847, Falkland Islands (Islas Malvinas), 52°00' S, 56°36' W, R/V Eltanin, cruise 7, sta. 558, 14 Mar. 1963, 646–845 m. Paratype (Figures 6–8), USNM 898774, Falkland Islands (Islas Malvinas), W of Beauchene Island, 53°06'S, 59°24'W, R/V Eltanin, cruise 6, sta. 340, 12 Mar. 1962, 567–578 m.

Type Locality: Falkland Islands (Islas Malvinas), 52°00' S, 56°36' W, R/V Eltanin, cruise 7, sta. 558, 14 Mar. 1963, 646–845 m.

Other Material Examined: Two specimens (USNM 887765) (Figures 9–10) collected off Cape Horn, 56°06' S, 66°19' W, R/V Eltanin, cruise 9, sta. 740, 18 Sep. 1963, 384–494 m.

Remarks: The paratype (Figures 6–8) is a slightly smaller specimen, otherwise in all respects it is similar to holotype. One additional juvenile, a dead-collected specimen (shell length = 8.1 mm) (Figures 9–10) is rather similar in shell sculpture and outline to the types, but

Table 1. Shell dimensions of the holotype and paratype of *Jerrybuccinum malvinense* new species, measurements in mm.

	Holotype	Paratype
Shell length	24.1	17.8
Body whorl length	15.1	12.0
Aperture length	8.3	6.0
Shell diameter	10.2	7.9
Siphonal canal length	4.3	3.6

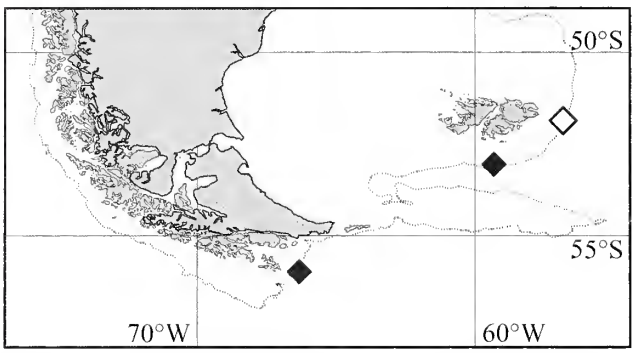


Figure 13. Geographical distribution of *Jerrybuccinum malvinense* new species. Dashed line indicates 500 m isobath. Symbols: ◊ = type locality, ◆ = other material examined.

differs in having a smaller protoconch (exposed height 1.12 mm vs 1.75 in holotype).

Distribution (Figure 13): The species is known off Falkland Islands (Islas Malvinas) at the depth 567–845 m and off Cape Horn in 384–494 m.

Etymology: The species is named after the type locality, Islas Malvinas (Falkland Islands.)

DISCUSSION

The radular characters undoubtedly place *Jerrybuccinum* in Buccinulidae, but the subfamilial allocation is not clear. The single cusp of the rachidian tooth and long basal projection of the lateral tooth suggest the affinities with the subfamily Cominellinae and particularly with the Antarctic species of "*Pareuthria*", i.e., *P. plicatula* Thiele, 1912, *P. innocens* (Smith, 1907), and *P. hoshiaii* Numanami, 1996 (see Numanami, 1996). Harasewych and Kantor (2004) observed that the Antarctic representatives of this genus differ markedly in radular morphology from those of Magellanic distribution, including the type species *Pareuthria plumbea* (Philippi, 1844), which has a tricuspid rachidian tooth. In any case, in contrast to the condition found in *Jerrybuccinum*, all representatives of Cominellinae have the bicuspid lateral teeth.

The shell in the new species does not have analogues among Antarctic and Subantarctic Buccinulidae. It shows some resemblance to representatives of Fasciolariidae, mostly due to the long and nearly straight siphonal canal and characteristic axial sculpture. Thus, on first approach, *Jerrybuccinum malvinense* appears to combine characters of both families.

ACKNOWLEDGMENTS

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A new species of *Paryphantopsis* (Gastropoda: Pulmonata: Charopidae) from Crater Mountain, Simbu (Chimbu) Province, Papua New Guinea

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ABSTRACT

Paryphantopsis bradleyi new species is described from a sub-montane forest near Crater Mountain Biological Research Station in Simbu Province, central Papua New Guinea. It is distinguished from its congeners by the combination of its large size and sharply pointed, non-overlapping periostracal processes that are retained to maturity. It shares similarities and is probably closely related to other large *Paryphantopsis* Thiele, 1928, that have angled to carinate shell margins with long periostracal processes and central and lateral radular teeth that have mesocones originating from the center of their basal plates. It appears that much of New Guinea's highly endemic terrestrial snail fauna remains to be discovered. It is imperative that the biodiversity of large groups of taxa is documented because this information will be crucial in efforts to preserve rapidly diminishing rainforest habitat.

Additional keywords: Terrestrial snail, pulmonate, rainforest, taxonomy

INTRODUCTION

The pulmonate snail family Charopidae Hutton, 1884, was once considered to be a minor component of the terrestrial molluscan fauna of New Guinea, in contrast to the group's spectacular radiations in the oceanic islands of the Pacific (Solem, 1983: 305). However, recent surveys in Papua New Guinea suggest that inadequate sampling, rather than low diversity, is the cause of the perceived paucity of charopid species in New Guinea (Slapcinsky, 2005). *Paryphantopsis* Thiele, 1928, the most diverse genus of charopids in New Guinea, consists of 26 described species distributed from Papua (Irian Jaya) to the Louisiade Archipelago and New Britain. Solem (1970) reviewed the 14 species of the genus then known, redescribing all species except those described or reviewed by van Benthem Jutting (1964). However, nearly half of this radiation has been described only recently (Slapcinsky, 2005, 2006; Slapcinsky and Lasley, 2007), and it is clear that continued sampling, especially in New Guinea's poorly sampled mountain ranges, will uncover many additional species. All *Paryphantopsis* species are restricted to single mountain ranges, each often supporting several *Paryphantopsis* species. The most widely distributed species, *Paryphantopsis yawii* Slapcinsky, 2005, ranges 50 km along the mountains of the East Cape Peninsula in extreme eastern Papua New Guinea. Synapomorphies in *Paryphantopsis* from the same mountain ranges suggest they have speciated on a fine geographic scale (Slapcinsky, 2005).

Most *Paryphantopsis* species occur in moist and mossy montane and sub-montane forests or at lower elevations in hill forest along stream valleys. Restriction to stable moist habitats and microhabitats might promote genetic isolation and rapid speciation in the group. Species of *Paryphantopsis* are unusual in being diurnally active (Slapcinsky and Lasley, 2007) and having reduced shells of approximately three whorls compared to at least four whorls in most other charopids. Whorl reduction is associated with enlargement of the shell aperture, modifications of the kidney, and reduced space in the pallial cavity for retraction of the visceral hump (Solem, 1970). All of these traits may make *Paryphantopsis* especially susceptible to desiccation if their moist forest habitats are altered. Besides shell whorl reduction and the associated changes in pallial organs, *Paryphantopsis* is also diagnosed by the following shell synapomorphies: protoconch sculpture of axial and spiral riblets that usually coalesce forming spiral rows of pits, growth lines that are accentuated with rib-like periostracal extensions that often bear processes at the shell margin, and shells that are not openly umbilicate.

Between 1990 and 1993, the Florida Museum of Natural History received a collection of terrestrial snails collected by Andy Mack and Debra Wright during studies at Crater Mountain Biological Research Station. This collection included a new species of *Paryphantopsis* which

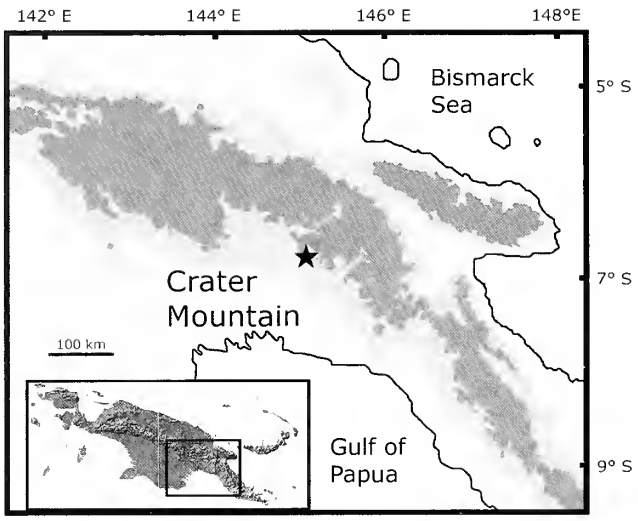
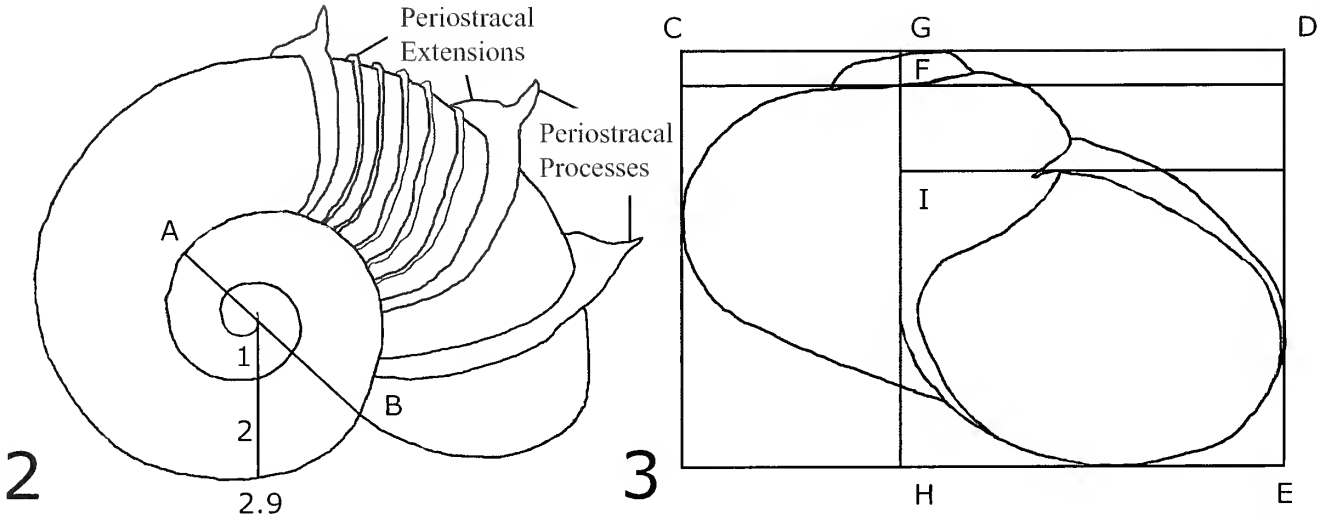


Figure 1. Map of eastern New Guinea showing the type locality of *Paryphantopsis bradleyi* new species

is described here. Crater Mountain Biological Research Station is located in southeastern Simbu Province, Papua New Guinea, approximately 78 km SSW of Goroka and 11 km E of Haia Village, at 6.72° S, 145.09° E (Figure 1). The research station is located on the southern slope of Crater Mountain, an arcuate chain of peaks reaching 3000 m or more in elevation and formed from an extensively eroded stratovolcano last active in the late Pleistocene or early Holocene (Mackenzie and Johnson, 1984). The topography of Crater Mountain is extreme, with vertical cliffs and frequent seismic activity, which, combined with ample rainfall results in numerous treefalls and landslides, lead to heterogeneous habitats and microhabitats. Geographic and habitat heterogeneity may contribute to the floristic richness of the area, the richest site known in New Guinea and among the richest in the world (Wright et al., 1997). A 1 ha plot contained 228 tree and liana species with no strongly dominant species. This floristically and geologically diverse site has not previously been sampled for terrestrial snails and is likely to sustain additional undiscovered species.

MATERIALS AND TEXT CONVENTIONS

Specimens were hand-collected or sifted from samples of leaf-litter. Live-collected animals were drowned and then preserved in 75% ethanol. Gross anatomical dissections were made under 75% ethanol using a dissecting microscope. Radulae were isolated from dissected buccal masses using a 5% sodium hypochlorite solution. Scanning electron micrographs of radulae were made using a Field Emission-SEM. Measurements were taken using an ocular micrometer. Whorl count was measured from the suture of the first whorl to the body whorl and fractions of a whorl were determined with the aid of a cardboard circle divided into ten equal parts of 36° (Figure 2, line 1–2.9). Spire width was the length of a straight line passing from the apertural edge of the suture through the middle of the apex to the opposite suture (Figure 2, line A–B). Shell width was the greatest width of the shell perpendicular to the shell axis (Figure 3, line C–D). Shell height was the greatest distance between the apex and the base of the aperture measured parallel to the shell axis (Figure 3, line D–E). Spire height was measured from the top of the body whorl to the apex of the shell (Figure 3, line F–G). Aperture width was the greatest distance from the columellar edge to the outer edge of the aperture (Figure 3, line E–H). Aperture height was measured from the suture to the base of the aperture, parallel to the shell axis (Figure 3, line H–I). Shell measurements are based on nine unbroken adults; ranges are followed by mean and standard deviation. The lengths of radular teeth



Figures 2–3. Diagrams of shell measurements. **2.** Whorl count (line 1–2.9), spire width (line A–B). **3.** Shell width (line C–D), shell height (line D–E), spire height (line F–G), aperture width (line E–H), aperture height (line H–I).

were measured from the top of the mesocone to the posterior edge of the basal plate. The widths of radular teeth were measured as the greatest width of the cusps, not the basal plate. The following abbreviations are used in figures of genital anatomy: AT = atrium, BC = bursa copulatrix, BT = bursa tract, EP = epiphallus, PE = penis, PP = penial pilaster, PR = penial retractor muscle, SO = spermoviduct, V = verge, VA = vagina, and VD = vas deferens. All specimens are deposited in the Florida Museum of Natural History, Gainesville (UF).

SYSTEMATICS

Family Charopidae Hutton, 1884
Genus *Paryphantopsis* Thiele, 1928

Type species: *Flammulina* (*Paryphantopsis*) *lamelligera* Thiele, 1928, by original designation.

Paryphantopsis bradleyi new species
(Figures 4–10)

Holotype: UF 378116 (dry shell), Papua New Guinea, Simbu Province, 78 km SSW of Goroka, 11 km E of Haia Village, Crater Mountain Biological Research Station, approximately 6.72° S, 145.09° E., 1100 m altitude, D. Wright, 6 Apr. 1992.

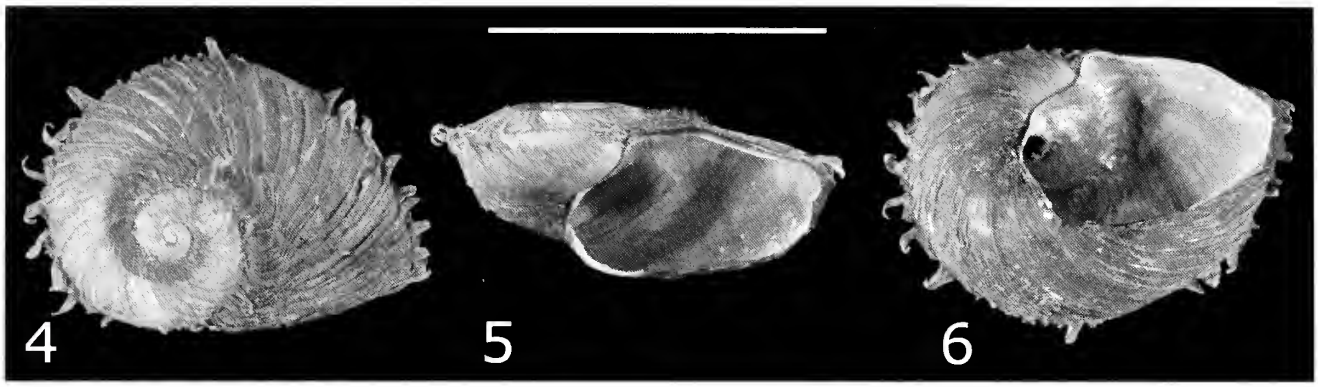
Paratypes: Type locality: UF 274062 (1 alcohol-preserved), UF 378115 (1 dry shell), 1100 m, D. Wright, 7 August 1991; UF 274059 (2 alcohol-preserved), UF 420747 (2 dry shells), 1350 m, D. Wright, 21 April 1992; UF 274061 (2 alcohol-preserved), 1100 m, D. Wright, 6 April 1992; UF 274057 (1 alcohol preserved), 1130 m, D. Wright, 18 March 1992; UF 179660 (1 alcohol preserved), 1160 m, D. Wright, 1 July 1990; UF 274060 (1 alcohol-preserved), A. Mack, 25 January 1993; UF 274058 (1 alcohol preserved), UF378114 (1 dry shell), A. Mack; UF 274056 (1 alcohol-preserved), A. Mack; UF 179657 (1 juvenile, alcohol-preserved), 1130 m., D. Wright.

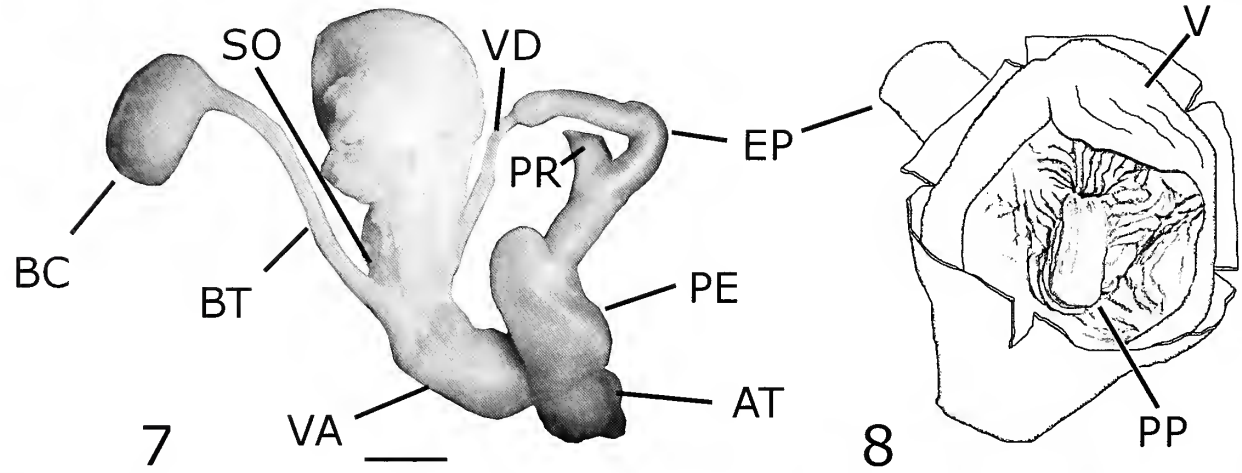
Description: Adult shell depressed; large for genus, 9.1–11.3 mm (10.3±0.8) in width and 4.1–5.9 mm (5.2±0.6) in height, with 2.8–3.1 (2.9±0.1) rapidly expanding whorls (Figures 4–6). Suture impressed and broadly channeled. Apical surface of whorls flattened between suture and periphery. Shell periphery angular to carinate above mid-point and rounded below, flattening abruptly basally. Spire flat or only slightly elevated, 0.0–0.3 mm (0.1±0.1) and narrow 3.4–4.1 mm (3.7±0.2) only 0.3–0.4 (0.36±0.02) of shell width. Teleoconch whorls do not descend or descend only slightly. shell height/diameter ratio 0.4–0.6 (0.50±0.04). Approximately 1.7 flattened protoconch whorls sculptured with about 12–17 rows of spiral pits that continue on teleoconch becoming elongate and less regular. These pits are obscured by thick periostracum on teleoconch but can be observed in aperture through translucent shell. Teleoconch whorls have growth lines accentuated with short periostracal extensions. Approximately every fifth extension longer and bearing a triangular process (approximately 1.0–1.5 mm long) at shell margin. These processes are retained to maturation. Protoconch whitish to tan. Teleoconch whorls brown; first 2.5 whorls darkest below suture and last 0.5 whorl dark throughout. A reflection of peristome completely covers umbilicus at all stages of growth. Aperture is ovate to almost quadrate and very large with an aperture-height to aperture-width ratio of 0.4–0.7 mm (0.63±0.10).

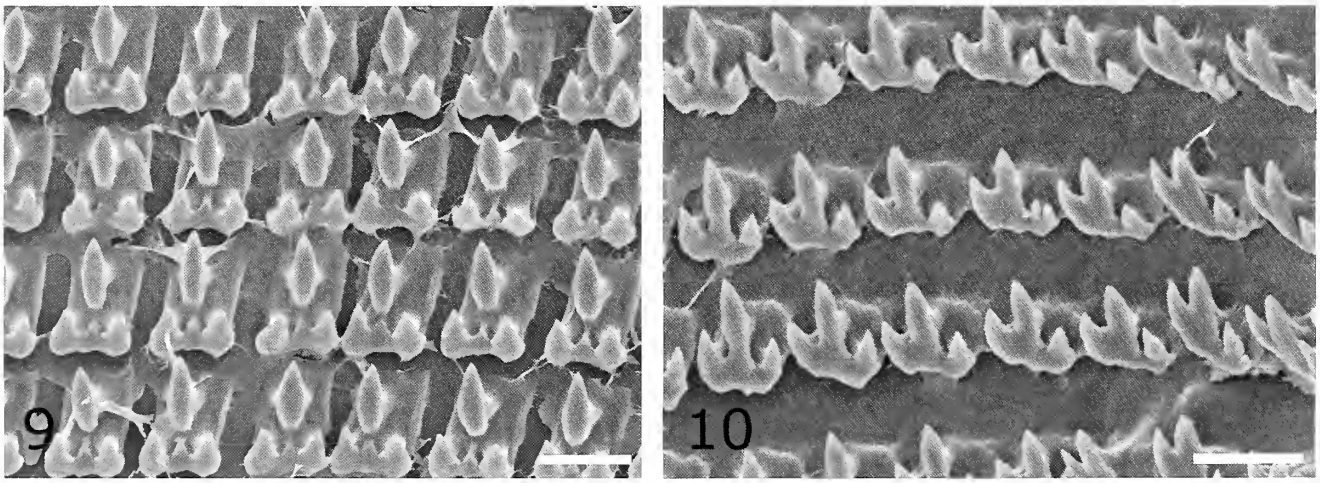
Epiphallus 3× diameter of vas deferens, only slightly inflated apically, widening gradually basally, folded approximately at mid-point, and does not bear an apical diverticulum (Figure 7). Penial retractor muscle short and robust, originating from diaphragm and inserting on epiphallus half way between epiphallar fold and junction with penis. Penis ovate and slightly inflated basally; epiphallus joins it laterally just below rounded apex. Penis width is 0.5× length and 2–3× width of epiphallus. Penis wall thin and smooth and interior of retracted penis containing large verge that, when unfolded, is bowl-shaped (Figure 8). Interior of verge is sculptured with tongue-shaped pilaster that extends from epiphallar opening to near base of penis. Atrium short and broad, with nearly same diameter as penis. Vagina relatively long, with about same length and only slightly narrower than penis. Free oviduct short, and with nearly same width as and poorly differentiated from vagina. Base of bursa tract narrow, its diameter only 0.3× diameter of free oviduct where they meet, rapidly narrowing to 0.2× diameter, and remaining narrow to junction with bursa copulatrix.

Central and first lateral teeth of radula are tricuspid, 9–10 μm wide, and 11–12 μm long (Figure 9). Mesocones of central teeth and lateral teeth tall and slender, projecting slightly beyond their basal plates, and originating from center of their basal plates rather than from a ridge on posterior edge of basal plate as in most other *Paryphantopsis* species. Ectocones of central teeth trigonal and symmetric. Ectocones and endocones of lateral teeth are trigonal and about 0.5× height of mesocones. Endocones and ectocones of lateral teeth nearly symmetrical, endocones very slightly larger but otherwise of similar shape to their ectocones. First 15 teeth to left and right of central row are similar to first lateral teeth, next two teeth on either side grade in shape and are difficult to classify as either lateral or marginal teeth. Last seven teeth clearly marginal and dorsoventrally compressed, 11–12 μm wide and 8–9 μm long (Figure 10). Endocones of marginals unicuspid and about 0.7× as tall as mesocones. Ectocones are unicuspid to irregularly multicuspid and much shorter, about 0.5× as tall as mesocones.

Habitat: All specimens were collected from sub-montane forest (Paijmans, 1976) between 1100 and 1350 m elevation in leaf litter and on live leaves especially of Zingiberaceae within 1 m of the ground. Vegetation







Figures 4–10. *Paryphantopsis bradleyi* new species. **4–6.** Photographs of shell, holotype UF 378116, diameter 11.3 mm. Scale bar = 10 mm. **7.** Photograph of genitalia, UF 274062. Scale bar = 1 mm. **8.** Drawing of penis interior, UF 274062. **9–10.** Scanning electron micrograph of radula, UF 274062. Scale bars = 10 m.

at the type locality consisted of mature uneut forest with a few small patches of late secondary growth from abandoned gardens. Mean annual rainfall is 6400 mm and is evenly distributed throughout the year. Diurnal temperatures are 15–28° C. The area's soils range from dark brown loam to orange clay with variable soil nutrients including soil ealcium that ranges from 270 to 1560 ppm (Wright et al., 1997).

Etymology: This patronym honors botanist Ted Bradley, Santo Domingo de Heredia, Costa Rica (retired from George Mason University, Fairfax, Virginia) my friend, teacher, and field companion who encouraged my interest in taxonomy and introduced me to the rich and underreported diversity of the tropics.

Remarks: *Paryphantopsis bradleyi* new species is one of the largest species in the genus and is similar in size only to *P. louisiadarum* (Möllendorff, 1899) and *P. globosa* (Hedley, 1890), both of which differ in having shells with rounded margins that lack periostracal processes. *Paryphantopsis bradleyi* appears similar to species that have shells with angulate to carinate margins and that bear periostracal processes that are retained in adults: *P. corolla* Slapcinsky and Lasley, 2007, *P. elegans* (Fulton, 1902), *P. fultoni* (Coen, 1922), *P. lamelligera* (Thiele, 1928), *P. lebasii* Slapcinsky, 2005, *P. yawii* Slapcinsky, 2005, and *P. yelensis* Slapcinsky, 2006. The periostracal processes of *P. elegans*, *P. fultoni*, and *P. yawii* overlap, forming a continuous serrated edge at the shell margin, while the periostracal processes of *P. bradleyi*, *P. lamelligera*, and *P. yelensis* each taper to a point, unlike the rounded processes of *P. lebasii*. Of the species for which the genital anatomy is known, *P. bradleyi* is similar to *P. lamelligera*, *P. lebasii*, and *P. yawii* in lacking an apical diverticulum on the epiphallus, unlike *P. corolla* and *P. yelensis*. The mesocones of both the central and lateral teeth of the radula of *P. bradleyi* join the basal plate near its center as in *P. lebasii*, *P. yawii*, and *P. yelensis*, and unlike *P. corolla*.

DISCUSSION

Unlike that of many other taxa, species diversity of terrestrial snails has been considered to be low in tropical rainforests (Solem, 1984). However, recent surveys have demonstrated that terrestrial snails are often diverse in tropical rainforests (Winter and Gittenberger, 1998; Schilthuizen and Rutjes, 2001) and it appears that low abundance and sampling intensity are the reasons for perceived low diversity of snails in rainforests. Lack of sampling is particularly troubling because rapid deforestation is leading to the extinction of narrowly endemic snail species in many tropical forests (Emberton, 1995; Emberton et al., 1997). Unfortunately, non-marine mollusks appear to be particularly prone to extinction, constituting an alarming 42% of the recorded extinctions of animal species since the year 1500 (Lydeard et al., 2004). Much more of this loss may go unreported because terrestrial snails receive relatively little taxonomic study in relation to their diversity. Indeed, there are approximately 24,000 described species and an estimated 11,000 to 40,000 undescribed species (Lydeard et al., 2004). The land snail fauna of New Guinea appears to be especially diverse but few of its mountain ranges have ever been surveyed for most invertebrate groups. These mountains support diverse and highly endemic snail faunas that are only now being discovered (Slapcinsky, 2005). New Guinea sustains the largest tracts of tropical broadleaf forest remaining in Australasia and the third largest on the planet after the Amazon and Congo forests (Brooks et al., 2006). More than 71% of Papua New Guinea is forested and 57% of this forest is commercially valuable and globally imperiled lowland rainforest (Shearman et al., 2008). These resources will come under increasing commercial and developmental pressures as other forests in the region are exhausted. Already, the rate of deforestation in Papua New Guinea is higher than previously believed, and is accelerating, and if these rates continue it is estimated that 83% of the country's forests will be cleared or degraded by 2021 (Shearman et al., 2008). The loss of these forests will result in the extinction of endemic species dependant on forest habitat, many before they are ever discovered. Future efforts to preserve rapidly dwindling forests will depend on documentation of their rich biota.

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Juan José Parodiz (1911–2007): obituary and bibliography

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INTRODUCTION

Every now and then, we meet an individual who possess knowledge not only in depth in a given subjeet, but also across several fields of knowledge. Often, we are not aware of these many interests of this Renaissance person. I knew Juan José Parodiz as a malacologist and eurator emeritus at the Carnegie Museum of Natural History. It was after he died that I learned much more about him and his many accomplishments.

José, as he preferred to be called, passed away on 4 September 2007 at the age of 95 years. He was born in Buenos Aires, Argentina, on 21 December 1911. He was one of the last of the classieally trained malacologists, brought up in an era before the widespread use of computers, molecular biology, and cladistic analyses.

THE EARLY YEARS

José was the son of Mercedes Gonzalez Parodiz. José never knew his father and his mother died when he was 8 years of age. He and his younger sister Avelina then went to live with an aunt, Avelina Andrea Parodiz, whom they regarded as their mother. She was employed as a postmaster in Buenos Aires. This may explain José's later interests as a philatelist. José had three other aunts, and one of them, Juana Pabla Parodiz, made him promise that he would look after them until they died. This promise, which he honored, was to play a central role in his life.

José went to work at the Museo Argentino de Ciencias Naturales "B. Rivadavia" (MACN) in 1927. He was 16 years old. José mentioned that his "mother" wanted him to be a lawyer, however, this profession held no interest for him. On the other hand, what sparked his interest in natural history is unknown.

MUSEO ARGENTINO DE CIENCIAS NATURALES (1927–1952)

At the Museo Argentino de Ciencias Naturales, José went to work in the section of invertebrate biology. He worked under two men that he held in great esteem, Dr. Martin Doello Jurado and Alberto Careelles. Doello Jurado was the director of the museum. Carcelles, like José, eame to the museum with no formal training. José was trained by Doello Jurado and Carcelles. Carcelles went on to become an eminent malacologist working with marine mollusks. José's first paper was co-authored with him.

Parodiz was involved in oceanographic expeditions in the South Atlantic and the Southern Ocean. These expeditions occurred in 1938 and 1939. He spent time on the ships ARA Comodoro Rivadavia and ARA Baiha Blanca (Patagonia, Tierra del Fuego, Isla de los Estados, and the Magallanes Strait). On these expeditions, not only mollusks, but other invertebrates (and fish) were collected. The collections included dredged material as well as shore-collected specimens.

After José started his work in Recent invertebrates, he was eventually appointed the head of invertebrate paleontology (1940-1952) at MACN. Throughout his career, he would continue to study both fossil and Reeent mollusks of South and North America.

While in Argentina, José was a member of the Asociación Argentina de Ciencias Naturales. He served as seeretary from 1945–1950 and resigned from the association in 1952. Though José was mainly involved with mollusks, he had an interest in invertebrates in general. There are unpublished manuscripts of talks that he gave over the radio. Two of these talks were titled "Sponges" and "Crustaceans of economic valne". These talks were broadcast from 1942 to 1944.

José also worked at the Estación Hidrobiológica de Puerto Quequén (a part of MACN). This was a research facility that was started by Doello Jurado. José was also an assistant in Geology and Paleontology at the University of Buenos Aires (1930–1933). With whom he worked at the University is unknown. From 1935 through 1945, José eollaborated with Egidio Feruglio, a geologist, on identifying fossil mollusks.

Upon the retirement of Doello Jurado, the MACN was directed by Agustín Eduardo Riggi. José and Riggi did not get along. José felt that Riggi was politically motivated and did not possess the same qualifications that Doello Jurado brought to the director's position.

[1] Researeh Associate

There was a good deal of animosity in their relationship that would come to play in decisions that José made in the future.

While José worked at the MACN, he met many eminent scientists, especially from the United States. Among the scientists were Fritz Haas, Henry A. Pilsbry, and Waldo L. Schmitt. These individuals encouraged José to visit and study in the United States. José planned a visit to the United States with Schmitt's assistance; however, shortly before he was to make the trip, he cancelled it due to a political coup that was unfolding in Argentina.

American museums hold many of the type specimens of South American mollusks and José wanted to study them. In 1949, José applied for a fellowship from the John Simon Guggenheim Memorial Foundation. He was awarded a fellowship and in 1950 he spent six months in the United States. José conducted the majority of his research at the National Museum of Natural History (Smithsonian Institution), and the remainder at the Academy of Natural Sciences (Philadelphia) and the Museum of Comparative Zoology (Harvard University).

Prior to leaving for the United States to conduct his studies, José requested that he be kept on the payroll at the MACN. Riggi refused. José saved his vacation time from 1949–1950 so that he would have some paid time while in the Unites States. José was scheduled to spend a year in the United States. The staff at the Guggenheim Foundation allowed José to shorten his stay to 6 months due to his financial situation.

While in Washington, DC, José lived in housing arranged for by Waldo Schmitt. The landlady had a friend, Esther Elizabeth Sell, who worked as a secretary in the Treasury Department. She introduced Esther to José, and Esther and José became romantically involved. Prior to returning to Argentina, José promised Esther that he would return and that they would wed. Needless to say, Esther never thought that she would see him again.

During José's stay in the United States, he attended the annual meeting of the American Malacological Union (AMU; now American Malacological Society, AMS), which was held in Chicago. Years later, José was to serve as president of this group. During his visit to the Midwest, José collected Unionidae from the Meramec River near St. Louis. His notes indicate that he also visited the "Chicago Museum" (probably the Field Museum of Natural History) and the University of Michigan Museum of Zoology. At the AMU meeting, he met many of the most influential malacologists in the United States. From José's work during his fellowship, two papers were written (Parodiz 1950, 1962b).

José and Riggi had a contentious relationship before José went to the United States, which only worsened upon José's return to Argentina. This increased animosity was fueled in part by laudatory letters sent to Riggi by Schmitt, Clench, and Haas, people who José met or studied under in the United States. These letters described the high quality of José's scholarship. José's success in the United States put him under closer scrutiny by Riggi.

Upon returning to Argentina, José was informed that his last surviving aunt, Juana Pabla Parodiz, had passed away. She passed away the day José left New York City to return to Buenos Aires. He was no longer bound to his promise to help care for her. This release, along with a deteriorating relationship with his superior, and a woman that he loved in the United States, set in motion his next plan, one of permanently immigrating to the United States.

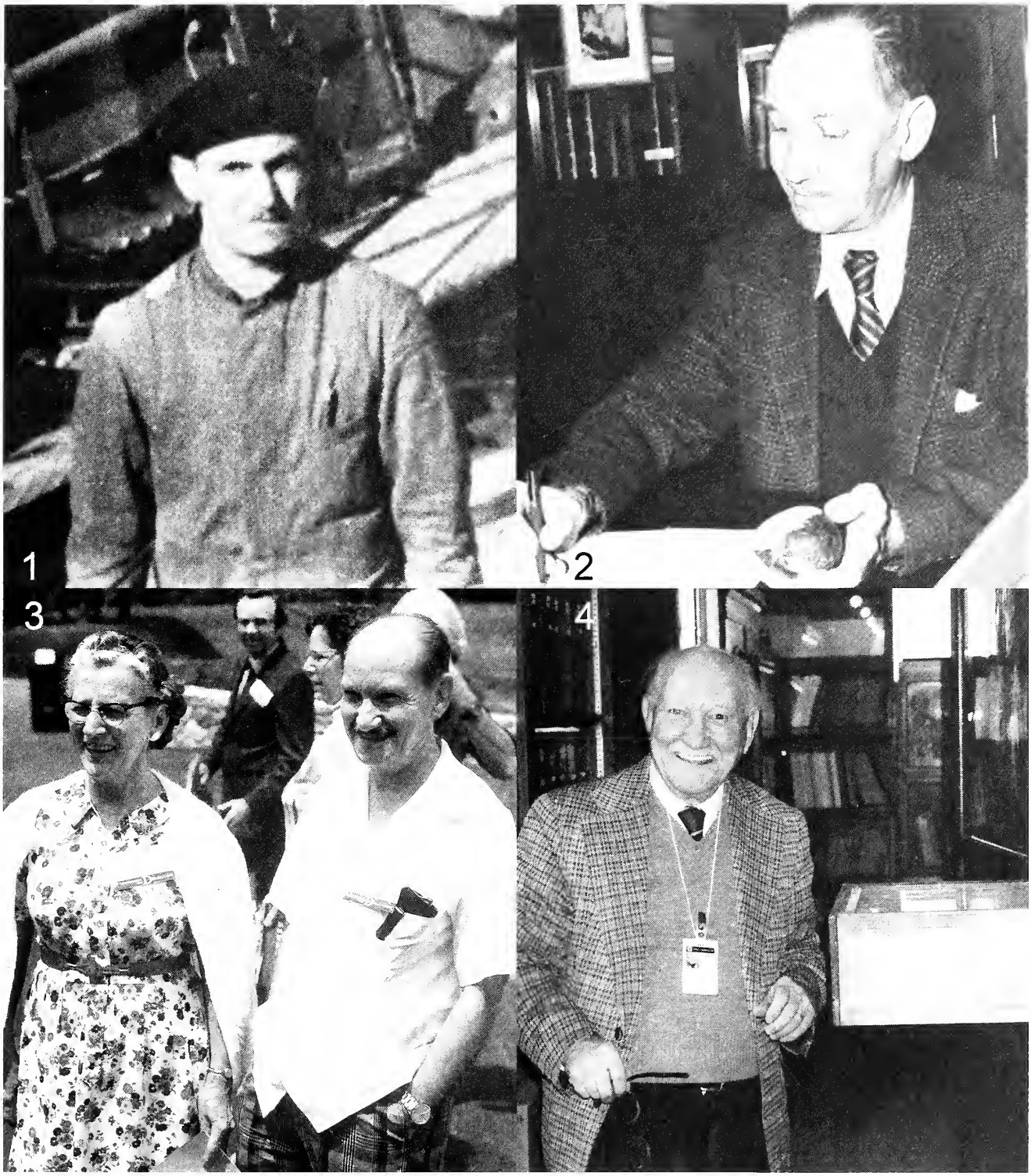
At this point I would like to address José's education. José was often addressed as "Doctor Parodiz"; however he appears to have had no formal academic degree. He joined the MACN at the age of 16 and was trained by Doello Jurado and Carcelles. He assisted someone at the University of Buenos Aires, yet there are no documents of what he did there, though he was fond of recounting his stories with the student theater ensemble. In a 1948 issue of Comunicaciones del Museo Argentino de Ciencias Naturales Serie Ciencias Zoológicas, the journal of MACN, the members of the staff are listed with their titles: "Dr.", "Prof.", and "Lic.". José's name is preceded by none of these designations. On his application for the Guggenheim fellowship, the section of the application which asks for the applicant's educational background was left uncompleted. Lastly, queries to staff at MACN resulted in no information regarding any formal training that José may have obtained (pers. comm., M. G. Quintana, 2007). We may draw then the conclusion that José had no academic degree and that his title as a doctor was a well-deserved honor bestowed upon him by his colleagues in recognition of his significant accomplishments.

CARNEGIE MUSEUM OF NATURAL HISTORY (1951–2007)

Once José decided to move to the United States, he needed to find a job. He wrote to contacts in the United States and Schmitt informed him of a curatorial position that was open at the Carnegie Museum of Natural History (CMNH) in Pittsburgh. This position had been vacant for a year. José applied for the position and was hired. He began his duties at the CMNH in 1952. Somewhat jokingly, he once said that no one else wanted the job due to the low salary.

José informed few colleagues in Argentina of his plans to move the United States; he was fearful of retribution by Riggi. José saved his vacation time, packed, and left for the United States. Upon learning of José's departure, Riggi attempted to have him fired to create a black mark on his record. Senior staff of MACN would not allow such an action.

Prior to settling in Pittsburgh, José returned to Washington. On April 26, 1952, he wed Esther Sell. Waldo Schmitt was José's best man. José then moved to Pittsburgh where he and Esther resided together until her death.



Figures 1–4. Juan José Parodiz. **1.** Aboard the Argentinean Navy research vessel the ARA Bahia Blanca in 1939. **2.** At CMNH in 1961. **3.** With wife Esther at the AMU meeting in 1973 (Newark, Delaware). 4. As Curator Emeritus in 1997, working in the mollusk collection at CMNH. Figures 1, 2, and 4 are from the archives of CMNH, figure 3 was from the collection of Robert Robertson, Curator Emeritus, Academy of Natural Sciences, Philadelphia.

José was a curator at the Carnegie Museum of Natural History from 1952 to 1980. He retired in 1981 and was active as a curator emeritus until his death in 2007. Upon arriving at CMNH, José developed a 10-year plan. Of this plan, he succeeded at some parts and never completed others.

One of José's plans was to reorganize the collection. This project extended well past the first ten years and was never fully realized. While the Unionoida and Gastropoda were reasonably well organized, the reorganization of the marine bivalves was not completed at the time of his retirement. The Sphaeriidae, most of which were transferred to the CMNH upon Victor Sterki's death (1933), were virtually untouched by José. José began a project of segregating type material from the general collection. He placed the type specimens in a separate cabinet. The majority of the type specimens that he overlooked were the types of Sphaeriidae in Sterki's collection. Only six lots of an estimated 900 lots were transferred to the type cabinet.

Another aim of José's 10-year plan was to maintain continued collaboration with malacologists in South America. This part of his plan succeeded remarkably well. He not only continued to undertake fieldwork in South America, but South American scientists came to the Carnegie to study its collection and to collaborate with José. This will be discussed in more detail later in this paper.

José sought to expand the molluscan collection at CMNH. He especially wanted to expand the collection of South American mollusks. The Carnegie already had a significant collection of South American unionoids collected by John Haseman in the 1920s. José embarked upon an exchange program with other museums, thus expanding the number of holdings at CMNH.

Prior curators at CMNH studied and published on various groups of Pennsylvania mollusks. Arnold Ortmann published on the unionids, Victor Sterki on the Sphaeriidae, and Stanley Brooks on the terrestrial Gastropoda. A comprehensive study on the freshwater gastropods was lacking. José planned to undertake such a study. Periodically, José worked on this study from when he proposed his 10-year plan until his retirement. He did not complete it. In 1956, he published a paper on one aspect of freshwater gastropods in Pennsylvania (Parodiz 1956d). In 1958, José published a list of the freshwater gastropods of Pennsylvania (Parodiz 1958c). In this paper, José indicated that this list was a prodrome of a more extensive manuscript, soon to be submitted for publication. On this point, José erred.

In the late 1970s, while collaborating on a study with C.R. Bristow, José mentioned that the director of CMNH was urging him to finish the study of the freshwater gastropods of Pennsylvania. In a letter to William J. Clench, he mentioned that he was waiting for Clench's monograph on the Pleuroceridae and posed several questions to Clench. Clench's monograph was published; still, José's manuscript remained just a manuscript. His 150-page manuscript on the freshwater gastropods remained unpublished. For whatever reasons, José invested significant time on this project. He conducted field studies, reviewed collections in museums, and corresponded with others. His work still remains in the manuscript stage and the Pennsylvania freshwater gastropod fauna still await a comprehensive study.

A few years after José arrived at CMNH, Waldo Schmitt offered him a position at the National Museum of Natural History. José declined the offer despite its larger salary and a larger collection at the National Museum of Natural History. There was one significant reason for José turning down the offer. The National Museum of Natural History was part of the Smithsonian Institution, an institution run by the United States government. Though José and Schmitt shared a close friendship, José did not want to entertain the possibility of finding himself, in the future, of answering to an eventual political appointee. He did not wish to be in the same position that he was in Argentina when Doello Jurado retired and was replaced by Riggi. José, who liked the director at CMNH, chose to pass up the benefits of a new position at the Smithsonian and remained in Pittsburgh, at a private institution.

In 1954, the journal Neotropica was launched. José was one of its founders. José remained on the editorial board from 1954 through 1972. In addition, José served as the North American contact for the journal. José was also active in the journal Malacologia. Initially, José was responsible only for translating abstracts into Spanish. Shortly thereafter, he was asked to join the editorial board. He served in this capacity from 1962–2006. Yet a third time, José was asked to be on the editorial board of a journal. This time, John Burch (in litteris, 1990) requested that José join the editorial board of Malacological Review. During his career, José also served as a reviewer for several other journals including The Nautilus and the Journal of Morphology.

José was responsible for the mollusk display at CMNH. This was developed in the early 1960s under the sponsorship of the Commonwealth of Pennsylvania. The exhibit, which is still extant, comprises six display cabinets, each approximately 0.9 × 1.2 m. The exhibit is titled "Sea Shells by the Seashore". The display cabinets are titled (1) How Shells are Named, (2) Shells and Such (Classes of Mollusks), (3) Eating and Moving, (4) How Mollusks Reproduce, (5) Scavenging in New England, and (6) Beach Combing in Florida. This exhibit was the subject of an article in the Carnegie Magazine (Parodiz 1962e).

José received only one governmental grant during his career. It was a National Science Foundation grant, awarded for the years 1961–1962, for the study of nonmarine mollusks of Argentina, Uruguay, and surrounding territories. José conducted field work (with Alberto Carcelles and Argentino A. Bonetto) during those two years, purchased the equipment that he needed, and concluded his work with 10% of his funding still intact. José refused to use the monies for other purposes. He

felt that he had completed the work and any remaining funds should not be used for other purposes but returned to the granting agency. José was hassled by a few colleagues for this action, so much so that he chose never again to apply for another grant. Among the papers that resulted from this grant are Parodiz (1963c, 1965b, 1966a), Parodiz and Bonetto (1963), and Parodiz and Hennings (1965).

José was parsimonious. He would combine visits to friends and family in Argentina with his field work. Since he knew about the political and economical fluctuations in South America, he was able to fund his work in South America from his museum budget. Even the director who hired José, Graham Netting, realized José's thriftiness. In a memo, Netting commented about this by writing "...since you rarely succeed in spending your entire Section budget..." (in litteris, 1969).

In 1962, Dean Putnam Jones of the University of Pittsburgh proposed to Chancellor Litchfield that José should be appointed as an adjunct member of the Graduate Faculty at the University of Pittsburgh. In a letter dated 2 January 1963, Litchfield informed José of his appointment as an adjunct member of the Graduate Faculty. José never acted in a professional capacity at the University. He felt that his responsibilities as curator were such as to preclude his serving in a professorial role at the University. As he put it in a memo to Craig Black (director of CMNH, 1975–1982), "I was convinced that I could not fulfill both efficiently, at the same time."

José, along with Gladys McCallum, founded the Pittsburgh Shell Club in 1965. The first meeting was on 27 March 1965, with 13 people in attendance. José was appointed councilor (advisor) of the club. The club published the Pittsburgh Shell Club Bulletin (1966–1979). José was a frequent contributor to the publication. In 1975, José was awarded the DuPont Trophy for his outstanding exhibit at the Pittsburgh Shell Club Show. The exhibit dealt with the evidence for evolution as seen in fossil and Recent mollusks. In 1977, José was elected an honorary member of the Pittsburgh Shell Club. The only other person so honored was William J. Clench of Harvard University.

While José spent most of his field time in South America, in 1976 he had planned a trip to Guatemala. Shortly before he was to leave, an earthquake there forced him to cancel his trip. Instead, he went to the Yucatan Peninsula (Mexico) and stayed with a friend, Dorothy Zapata. Her husband drove José around during his stay. For three weeks, José collected marine mollusks. In all, he visited ten stations. He published his catalog for this trip in the Pittsburgh Shell Club Bulletin (Parodiz 1979e). This paper includes a number of range extensions for mollusks found in the Caribbean biogeographic region.

In addition to pursuing his own research interests in South American malacology, José sought to help others. One such example involved Kenneth Boss who was studying the neotropical fossil Ampullariidae. In 1975, he started corresponding with José. Several letters later, Boss offered José co-authorship on a paper on which he was working. The paper was published in 1977 (Boss and Parodiz, 1977). Another example involved C. R. Bristow. Bristow was a British geologist working in South America. Bristow began his correspondence with José in 1972. Six years later, he suggested that they jointly publish a paper on the Tertiary non-marine mollusks of Ecuador. The paper was published in 1982 with Bristow writing the stratigraphic portion and José the taxonomic portion (Bristow and Parodiz 1982).

José hosted many South American malacologists at CMNH. Among them were Miguel Klappenbach (1963), A. Carcelles (several times, his longest visit being 8 months in 1965–66), Maria C. Dreher Mansur (1998), and A. Bonetto (1959, 1963). In addition, José aided researchers from all over the world with his knowledge of South American malacology, paleontology, and politics. Numerous students and researchers sought his opinion of their proposed studies in South America. José would advise them of localities that they should visit, museums where they would find specimens for study, and whom to contact while in South America. If it was appropriate, he would comment on the political situation that waited for them. José spoke with the authority of one who had an intimate working knowledge of South American museum collections, knowledge built over the decades from studying these collections personally. In an era before the Internet, before one could easily search for such information, José served this purpose.

José also aided others in their work with the North American fauna. Some examples include identifying (1) the gastropods in the stomach contents of box turtles (1955), (2) the freshwater gastropods from the Cheat River in West Virginia (1962), and (3) the freshwater gastropods from the Susquehanna River in Pennsylvania. Also, he provided comments to the authors of a new unionoid from the Mesozoic of Uruguay (1993).

An intriguing aspect of José's work were the forensic studies in which he was involved from 1953–1963. One such case involved a worm found in a can of chicken soup. José identified it as a blood vessel from a chicken. He did not specify whether it was an artery or a vein. Another case involved a can of food that had been imported and contained maggots. He determined that the maggots were not from the country where the product originated and thus absolved the company of any wrongdoing.

José maintained membership in a number of professional societies. These include the Malacological Society of London, Unitas Malacologica, the Paleontological Research Institution, Brazilian Society of Malacology (honorary member), the Malacological Society of Uruguay (honorary member), and as mentioned earlier, the Pittsburgh Shell Club and the Argentine Association of Natural Science. José was also a member of the American Malacological Union (later the American Malacological Society). He served as president of this organization from 1964–1965.

José was curator emeritus at CMNH from 1981–2007. He continued to publish during this period. His last publication dealt with a South American unionid (Parodiz and Morton, 2002).

José was honored on three occasions by fellow scientists. In 1998 he was awarded a "Diploma of Honor" for his life's work. This award was bestowed on him by the Brazilian Society of Malacology. In 1992, Balech and José published a history of the MACN. In 2001, along with his friend Balech, he was honored as an "Illustrious Researcher" of the MACN. Lastly and posthumously in 2008, José was acknowledged as "an esteemed colleague, a distinguished malacologist, and a warm-hearted friend" by the American Malacological Society.

OTHER INTERESTS

José's views of evolution paralleled, to a degree, those of the Jesuit priest and paleontologist Teilhard de Chardin. José felt there was no incompatibility with a belief in God and natural selection. He did feel that evolution had some predestined direction and yet the forces of natural selection were evident wherever one looked in the natural world. His views are spelled out in *The Conceept of the Speeies* (Parodiz, 1977d).

José had a fascination with the explorations of Charles Darwin in South America. He felt there was some confusion regarding Darwin's travels in South America. In 1981, he published *Darwin in the New World* (Parodiz 1981). In this work, José described Darwin's journeys throughout the South American continent and provided historical commentary on what was transpiring in South America at that time.

José was a passionate philatelist. He was a member of the American Philatelic Society, the American Topical Association, and its chapter that dealt with biology on stamps. In addition to his memberships and collecting activities, he also wrote articles about stamps. In the Pittsburgh Shell Club Bulletin, Parodiz contributed three articles dealing with shells on stamps (Parodiz 1972e, 1973g, 1974c). He published six articles in Biophilately dealing with subjects such as cowries (Parodiz 1977i), biogeographic zones (Parodiz 1997b), and zoological nomenclature (Parodiz 1976d). As noted below, Parodiz enjoyed reading novels. In *Topieal Times*, he wrote articles about two literary figures that appeared on stamps, Edith Wharton and Thornton Wilder (Parodiz 1980c, 1998a).

José enjoyed reading novels. His friends describe him as well read. His favorite author was Victor Hugo. In addition, José was fond of Henry James, William Faulkner, Somerset Maugham, John Steinbeck, and Edith Wharton. At the time of his death, José was working on his own novel. I do not know the subject matter or how close to completion it was.

José had an excellent command of Spanish and English, both written and spoken. He also had familiarity with French, Portuguese, Italian, and Latin. José was a member of El Club Español de Pittsburgh—Pittsburgh's local club for Spanish-speaking people. One of my patients, who was born in Spain and was a member of the club, said that her sons were fascinated by José's stories and the talks that he gave at the club. He spoke about his work at CMNH, his trips to South America, and his work there. The Club honored him by awarding him a 'Diploma de Honor al Mérito'.

José never left his Argentinean culture behind. José brought a love for dance to the United States. José enjoyed the tango. In a letter to Anne LaBastille Bowes of Cornell University, he wrote "I have the tango in my chromosomes" (in litteris, 1969). In addition to maintaining his Argentinean culture, José also embraced an interest in opera and the symphony. On one occasion, he and Esther took a train to New York City to attend a musical performance, returning by train that same day. This was on of the ways that the Parodiz's could enjoy a full life on José's modest income.

THE LATTER YEARS

Esther Sell Parodiz passed away 22 February 2000. She and José had been married for 48 years. Their relationship was a close one. José's pastor, Reverend Eric Riesen, said that one did not speak of Esther or José but of Esther and José.

José continued to live in their Pittsburgh home after Esther's death. In 2007, he moved to Luther Crest Retirement Community in Allentown, Pennsylvania. José said that his advanced age was making it difficult for him to maintain his house. He enjoyed his new home and was very popular with the other residents. José's outgoing personality allowed him to fit in right away. In a letter to his former pastor, Rev. Riesen, José mentioned that one of the great pleasures of Luther Crest was its wonderful library.

On September 3rd, 2007, five months after moving, José took ill. He was taken to the hospital where he was diagnosed as having a heart attack (acute myocardial infarction). It was determined that he was not a candidate for aggressive therapy. He passed away the next day. José and Esther are interred in a cemetery on the grounds of Saint Peters Union Church, in Macungie, Pennsylvania, a site not far from where Esther grew up and José spent his final months.

Few people knew the breadth and depth of José interests. May of us knew one or two aspects of his life. Many of us came to realize his protean interests only when we discussed his life at his memorial service. I for one regret not having gotten to know José in the broader sense. As his friend S. Alan Boals put it, "Despite his long life, we feel cheated. We all would like a few more hours with José to have one last discussion on some topic of mutual interest." I for one regret José's passing for I would like to have many such discussions. I regret in having gotten to know him so well in death and not in life.

TAXA NAMED IN JUAN JOSÉ PARODIZ'S HONOR

Clathurella parodizi Figueiras and Broggi, 1976
Diplodon parodizi Bonetto, 1962
Epiphragmorphora parodizi Fernandez and Rumi, 1984
Opsiphanes mutates parodizi (Lepidoptera: Rhopalocera) Bristow, 1991
Parodizia Medina, 1959
Potamolithus parodizi Morton, 1986
Siphocypraea parodizi Petuch, 1994
Spixinella parodizi Hylton-Scott, 1952
Strophocheilus parodizi Klappenbach and Olazarri, 1965
Trophon parodizi Pastorino, 2005

TAXA DESCRIBED BY JUAN JOSÉ PARODIZ

New Genera and Subgenera

1. *Araucania* new genus Parodiz, 1954
2. *Astroborus* new name Parodiz, 1949
3. *Austrodiseus* new name Parodiz, 1957
4. *Calliostoma (Tropidotrochus)* new subgenus Parodiz, 1977
5. *Cyclodontina (Burringtonia)* new subgenus Parodiz, 1944
6. *Odontostomus (Ventania)* new subgenus Parodiz, 1940
7. *Paleoaneulosa* new genus Parodiz, 1969
8. *Paleobulimulus* new genus Parodiz, 1949
9. *Protoglyptus (Rimatula)* new subgenus Parodiz, 1946
10. *Protoglyptus (Obstrussus)* new subgenus Parodiz, 1946

New Species, Subspecies, "Forms," and "Varieties"

1. *Adelopoma paraguayana* new species Parodiz, 1944
2. *Araucania twomeyi* new species Parodiz, 1954
3. *Bulimulus corderoi* new species Parodiz, 1962
4. *Bulimulus moci* new species Parodiz, 1962
5. *Bulimulus (Lissoacme) ameghinoi madrynensis* new subspecies Parodiz, 1944
6. *Bulimulus (Seansicochlea) catamarcanus* new species Parodiz, 1956
7. *Bulimulus (Scansicochlea) hyltonscottae* new species Parodiz, 1956
8. *Bulimulus (Seansicochlea) rudiseulptus* new species Parodiz, 1956
9. *Bulimulus (Seansicochlea) strobeli* new species Parodiz, 1956
10. *Calliostoma (Tropidotrochus) jayae* new species Parodiz, 1977
11. *Cassis ketteri* new species Parodiz and Tripp,1993
12. *Chilina stenostylops* new species Parodiz, 1963
13. *Crepidula aeuleata fortis* new variety Parodiz, 1939
14. *Cyclodontina (Scalarinella) nattkemperi* new species Parodiz, 1944
15. *Diplodon transandinus* new species Parodiz, 1963
16. *Diplodon (Ecuadorea) bristowi* new species Parodiz, 1982
17. *Drymaeus hyltoni* new name Parodiz, 1957
18. *Drymaeus lynchi* new species Parodiz, 1946
19. *Drymaeus megastomus* new species Parodiz, 1962
20. *Drymaeus pereirai* new species Parodiz, 1958
21. *Drymaeus poecilus triciuetus* new subspecies Parodiz, 1962
22. *Drymaeus rehderi* new species Parodiz, 1962
23. *Drymaeus waldoschmitti* new species Parodiz, 1962
24. *Epiphragmophora birabeni* new species Parodiz, 1955
25. *Epiphragmophora feruglioi* new species Parodiz, 1969
26. *Epiphragmophora villavilensis* new species Parodiz, 1955
27. *Eriphyla miraflorensis* new name Parodiz, 1969
28. *Humboldtiana edithae* new species Parodiz, 1954
29. *Lioplacodes bolivianus* new name Parodiz, 1969
30. *Lioplacodes feruglioi* new species Parodiz, 1969
31. *Littoridina vianai* new species Parodiz, 1960
32. *Lymnaea klappenbaehi* new species Parodiz, 1969
33. *Lyrodes doellojuradoi* new species Parodiz, 1960
34. *Neocorbicula stelzneri* new species Parodiz, 1969
35. *Neopetraeus stelzneri conispirus minuta* new form Parodiz, 1948
36. *Neopetraeus stelzneri hybrida* new form Parodiz, 1948
37. *Neopetraeus stelzneri nonogastanus* new form Parodiz, 1948
38. *Neopetraeus stelzneri peristomatus paraconispirus* new form Parodiz, 1948
39. *Neopetraeus stelzneri tinogastanus* new form Parodiz, 1948
40. *Neopetraeus stelzneri seaber* new form Parodiz, 1948
41. *Neritina loyolaensis* new species Parodiz, 1982
42. *Odontostomus fasciatus tenuiseulptus* new subspecies Parodiz, 1962
43. *Odontostomus weyenberghi minor* new variety Parodiz, 1939
44. *Odontostomus (Sealarinella) cordovanus striatus* new variety Parodiz, 1939
45. *Odontostomus (Spixia) doellojuradoi* new species Parodiz, 1941
46. *Odontostomus (Spixia) doellojuradoi minor* new variety Parodiz, 1941
47. *Odontostomus (Spixia) columellaris* new species Parodiz, 1941
48. *Odontostomus (Spixia) holmbergi* new species Parodiz, 1941
49. *Odontostomus (Spixia) tucumanensis* new species Parodiz, 1941
50. *Paleoaneulosa kennerleyi* new species Parodiz, 1982
51. *Paleoaneulosa patagonica* new species Parodiz, 1969
52. *Paleobulimulus eoeenieus* new species Parodiz, 1949
53. *Peronaeus izozoensis* new species Parodiz, 1947
54. *Peronaeus (Lissoaeme) puntanus* new species Parodiz, 1947
55. *Peronaeus (Lissoacme) reedi* new species Parodiz, 1947

56. *Peronaeus (Lissoacme) torallyi avus* new variety Parodiz, 1947
57. *Peronaeus (Lissoaeme) torallyi corrugatus* new variety Parodiz, 1947
58. *Peronaeus (Lissoacme) torallyi nigrumbilicatus* new variety Parodiz, 1947
59. *Physa wielmanni* new species Parodiz, 1961
60. *Plectostylus argentinensis* new species Parodiz, 1951
61. *Plekocheilus (Eurytus) ameghinoi* new name Parodiz, 1962
62. *Pomaeea (Effusa) pattersoni* new species Boss and Parodiz, 1977
63. *Pomacea (Pomacea) prourceus* new species Boss and Parodiz, 1977
64. *Potamides ehaliana* new species Parodiz, 1969
65. *Potamolithus felipponei coneordianus* new subspecies Parodiz, 1966
66. *Potamolithus peristomatus misionum* new subspecies Parodiz, 1966
67. *Protoglyptus curamalalensis* new name Parodiz, 1957
68. *Protoglyptus deletangi* new species Parodiz, 1946
69. *Protoglyptus punctustriatus* new species Parodiz, 1946
70. *Protoglyptus (Rimatula) minutissimus* new species Parodiz, 1962
71. *Pyrgulifera selmena* new species Parodiz, 1969
72. *Siphoeypraea trippeana* new species Parodiz, 1988
73. *Strophocheilus (Megalobulimus) avus* new species Parodiz, 1949
74. *Taphius walteri* new species Parodiz, 1969
75. *Thaumastus patagonicus* new species Parodiz, 1946
76. *Triphora medinae* new species Parodiz, 1955
77. *Valvata windhauseni* new species Parodiz, 1961

PUBLICATIONS

This bibliography for Juan José Parodiz is probably incomplete. The archives, at CMNH, of José's activities contain many manuscripts. It is uncertain whether they were published. Occasionally, one contained a notation indicating that it was published, but not where or when it was published. When enough information was present, a search for the publication was made. Most attempts to find these publications were unsuccessful. Parodiz was involved with the Treatise of Invertebrate Paleontology. He was asked to write for the volume on the Gastropoda, specifically the parts dealing with the Bulimulidae, Orthalicidae, Odontostomidae, and Strophocheilidae. It is uncertain whether he completed this task as the volume was never published. In the decades of the 1950s and 1960s, José published a number of articles in *Enciclopedia Barsa*, a Spanish encyclopedia published by the *Encyclopedia Britannica*. While he is listed as a contributor in this work, specific articles are not attributed to a particular contributor. José did not maintain a list of the article that he wrote. Aside from these caveats, this can be considered a complete bibliography of José's publications in both the scientific and popular press.

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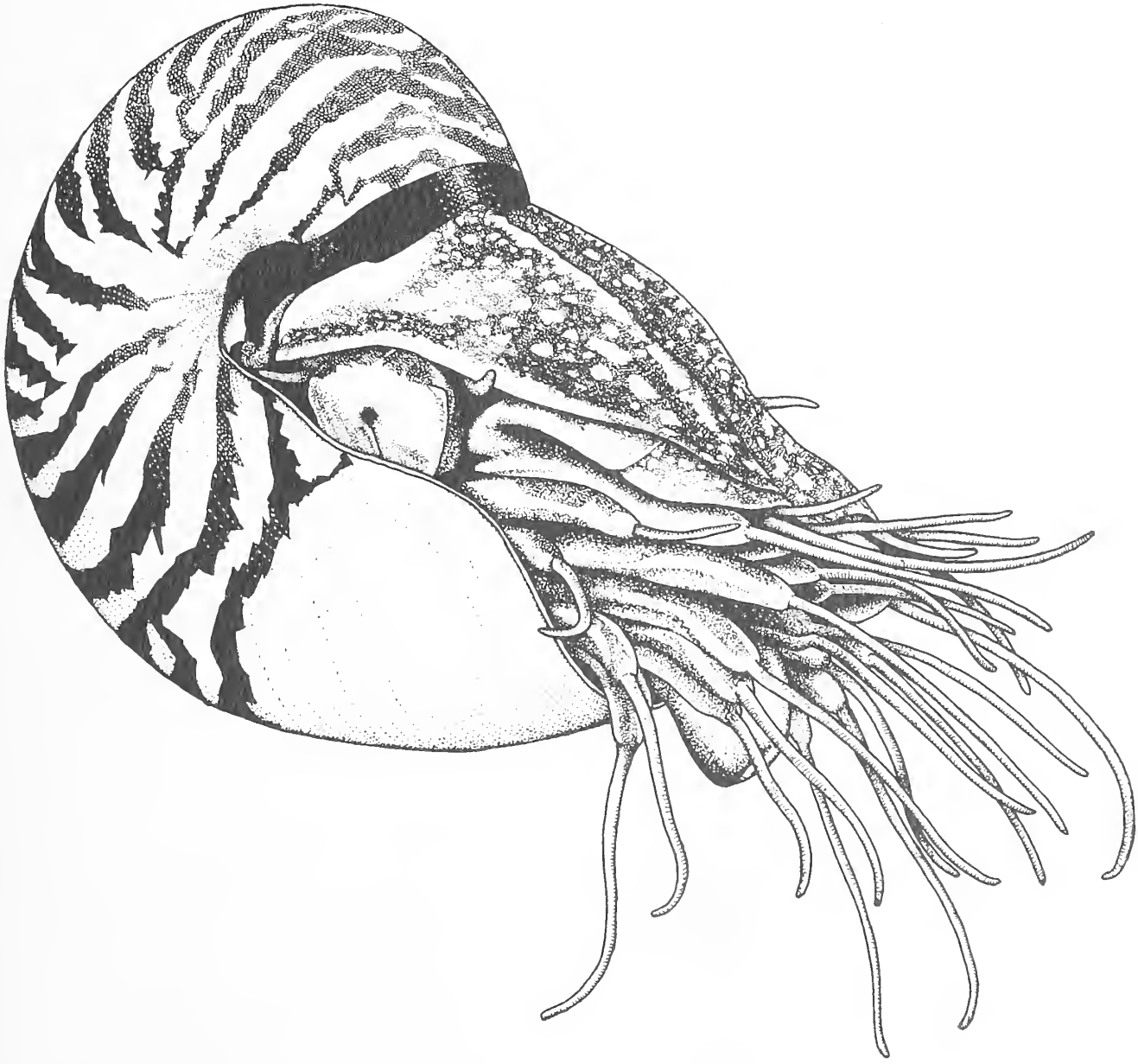

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Proceedings of the Symposium "Neogastropod Origins, Phylogeny, Evolutionary Pathways and Mechanisms" held during the 2007 World Congress of Malacology, Antwerp, Belgium, 15–20 July 2007

Guest Editors M.G. Harasewych and Ellen E. Strong

Preface

By any measure, neogastropods are an extraordinary example of evolutionary success. They appeared abruptly, at least in recognizable form, during the Albian (100 mya), and radiated rapidly at a variety of taxonomic levels to become the dominant predatory gastropods in benthic marine communities from the tropics to the poles, from intertidal depths to the abyssal plain. Several groups have further extended their range into the ocean trenches and into fresh water.

These animals have been studied extensively, and from numerous perspectives. Their economic importance is considerable, both as a harvestable food source and as predators of other commercially important mollusks. Neogastropod glandular secretions have also been of significant importance through the ages. Production of Tyrian Purple from hypobranchial gland secretions of muricids by the Minoans and Phoenicians has been traced to the 20^{th}–18^{th} centuries BC. In the present day, conotoxins, produced by the venom glands of toxoglossans, are being extensively studied for proven and potential biomedical applications.

Phylogenetic studies on neogastropods abound at a variety of taxonomic levels. Because of the rapid proliferation of lineages, each with tendencies to modify organ systems in parallel, most major groups are well characterized morphologically, yet precious few characters have been identified that support relationships within and between them. The absence of congruent patterns of character distribution in major organ systems has confounded initial attempts at phylogenetic inference based on morphological characters. More recent studies using DNA sequences of nuclear and mitochondrial genes have also produced contradictory or equivocal results, while analyses of datasets combining molecular and morphological characters have fared only slightly better.

Despite considerable and concerted research effort spanning decades, there are few questions that can yet be answered with any degree of confidence. When it comes to the most basic questions, we know remarkably little about neogastropods. Such questions as what? when? where? how? and why? still intrigue us. When rephrased in the language of modern biology, they become questions of monophyly, sister taxa, synapomorphies, fossil record, evolutionary rates, biogeography, as well inquiries that call into question our understanding of basic evolutionary and genetic mechanisms. Answers to many of these questions still evade us.

The first workshop focusing on the systematics, phylogeny and biology of the Neogastropoda was convened in Menfi, Italy (June 14–18, 2000), and followed by workshops on neogastropods at the Smithsonian Marine Station at Fort Pierce, Florida (August 4–13, 2004) and the Smithsonian Tropical Research Institute at Naos, Panama (January 29–February 13, 2006). A fourth workshop will be hosted by Centro Nacional Patagónico (CENPAT) in Puerto Madryn, Argentina (November 9–13, 2009).

The papers in this volume were presented as part of the symposium on Neogastropod Origins, Phylogeny, Evolutionary Pathways and Mechanisms that was convened during the 16^{th} World Congress of Malacology, held in Antwerp, Belgium, on August 16–17, 2007. Many of the participants in the neogastropod workshops were contributors to this symposium. The research presented here represents a broad spectrum of approaches to varied aspects of neogastropod evolution. We hope that these papers will shed light on some of the current questions, bring other unresolved issues into sharper focus and stimulate further research on this intriguing group of gastropods.

We are grateful to Prof. Dr. Thierry Backeljau and to the organizers of the Congress for the invitation to organize this symposium. Thanks are due to all of the participants who presented their research and to the many co-authors who were present, as well as to our many friends and colleagues who were unable to attend but nevertheless played a role in its success through their contributions.

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Morphology and development of the valve of Leiblein: Possible evidence for paraphyly of the Neogastropoda

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ABSTRACT

Neogastropoda are generally considered to be monophyletic, although their monophyly is usually challenged in molecular phylogenies. Such results suggest that serious reconsideration of the synapomorphies and autapomorphies defining the group is needed. One of the uncontroversial monophyletic groupings within the neogastropods is the superfamily Buccinoidea. This taxonomically rich clade lacks two out of three characters that are considered to be key autapomorphies of Neogastropoda, namely accessory salivary glands and the rectal gland. The only other autapomorphy that unites Buccinoidea with the rest of Neogastropoda is the valve of Leiblein. This study of the morphology of the valve of Leiblein of different neogastropods (two species of Raphitominae, Conidae, one of Muricidae, one of Nassariidae, one of Buccinidae, one of Cancellariidae, and one of Olividae) revealed its strong morphological heterogencity. Published and original data on the embryonic development of the valve in Buccinidae and Muricidae demonstrate that the valve originates from different sections of the anterior foregut. Preliminary data indicate that the homology of the valve of Leiblein within Neogastropoda is, at best, questionable. This casts further donbts on the monophyly of the Neogastropoda, whieh probably include at least two stems.

Additional keywords: Gastropoda, phylogeny, monophyly, alimentary system, development

INTRODUCTION

The origin and phylogeny of the Neogastropoda were the subjects of many publieations over the past decades. Several hypotheses on the sister groups were also proposed, including higher "mesogastropods" of the order Tonnoidea (Amaudrut, 1898; Graham, 1941, and more recently Riedel, 1994, 2000), and an "archaeogastropod" or primitive "mesogastropod" (Ponder, 1974). Morphological analyses of Strong (2003) suggested other possible affinities for the group, with the nearest relatives of Neogastropoda being Epitoniidae, Cypraeidae and Natieidae (Tonnoidea were not represented in these analyses). The molecular analysis of Colgan et al. (2007) found relationships between neogastropod families (either individually or as groups) and Turritellidae, Tonnoidea, Stromboidea, or Cypraeidae.

Since the publication of Golikov and Starobogatov (1988), mostly overlooked by western malacologists, the monophyly of the Neogastropoda was not contested. These authors suggested that Bucciniformii (to which they attributed majority of neogastropods, but also include Triphoridae) and Coniformii (in which they included Conoidea, Mitridae, Cancellariidae, and Pyrenoidea) originated independently, the former ones from Amberleyoidei while the latter from Turbinoidei. The idea that Conoidea (= Toxoglossa) stands well apart from the rest of the neogastropods was also supported by Sheridan, Van Mol, and Bouillon (1973) and Shimek and Kohn (1981).

Kantor (2002) summarized the major apomorphies of the Neogastropoda and eoncluded that they are monophyletic. Among recent morphology-based phylogenetic analyses, Strong (2003) and Ponder et al. (2008) supported the monophyly of the Neogastropoda. In Ponder et al. (2008) a Bayesian analysis of a combined morphologieal dataset and the molecular data also supported the monophyly of the Neogastropoda.

The monophyly of Neogastropoda has usually been challenged, albeit weakly, in molecular analyses (Harasewych et al., 1997; Colgan et al., 2000, 2003, 2007; Riedel, 2000; McArthur and Harasewych, 2003). More details of different molecular data analyses can be found in Colgan et al. (2007). It should be specifically noted that in a number of analyses the Tonnoidea were nested within the Neogastropoda.

A survey of existing theories and suggestions reveals that nearly every possible evohtionary scenario for the Neogastropoda, and nearly all possible relationships have already been proposed. A consensus has not yet been

achieved, and the situation is not becoming clearer with addition of more morphological and/or molecular data.

A major current problem is the incongruence between molecular and morphological analyses both in terms of the monophyly of Neogastropoda and the composition of the clade. The answer may lie in the erroneous interpretations of the synapomorphies and autapomorphies defining Neogastropoda. Taylor and Morris (1988) and, more recently, Kantor (2002) summarized and discussed in detail the autapomorphies of Neogastropoda. Three autapomorphies of neogastropods have been found so far: the presence of a second pair of salivary glands (accessory salivary glands, differing in morphology and histology from the primary salivary glands), the presence of a valve of Leiblein, and the presence of an anal, or rectal gland. It has been unanimously accepted that these three structures are homologous within the Neogastropoda.

One of the uncontroversial monophyletic groups within Neogastropoda is the superfamily Buccinoidea Rafinesque, 1815. This clade was considered as highly advanced by Kantor (1996), or the sister taxon to the rest of the neogastropods (Ponder and Lindberg, 1997). Buccinoidea lack both accessory salivary glands and a rectal gland, leaving the valve of Leiblein as the single remaining autapomorphy that is present in all major branches of the Neogastropoda.

The valve or pharynx of Leiblein is usually described as pear-shaped organ, consisting of a posteriorly directed cone-shaped protuberance that is enclosed in a chamber formed by the expanded walls of the anterior portion of the mid-esophagus (Brown, 1969). The protuberance, or flaps (*sensu* Fretter and Graham, 1962) are fringed with extremely long cilia that beat very languidly.

The major function of the structure is to prevent regurgitation of food from the more posterior part of the gut during the elongation of the proboscis. It reacts partially mechanically but also chemically—exposure to secretions of the digestive gland or stomach contents caused the flaps to close (Brock, 1936).

Surprisingly, the anatomy of the valve has not been studied extensively. In addition to the description of the valve of *Ilyanassa obsoleta* (Say, 1822) (Nassariidae) by Brown (1969), the valve was described in detail only for *Nucella lapillus* (Linnaeus, 1758) (Graham, 1941; Andrews and Thorogood, 2005). Despite these very limited data, the homology of the valve was never questioned. In light of the need to re-evaluate the phylogenetic value of autapomorphies for Neogastropoda, we undertook a comparative study of the valve in different branches of the Neogastropoda.

MATERIALS AND METHODS

Material for this study was collected in a number of localities; details are given in the corresponding descriptions for each species. For most species, the valve together with parts of anterior and mid-esophagus were dissected out from the body prior to fixation, then fixed in 4% formalin or 75% alcohol. In the laboratory, the valves were dehydrated and embedded in Paraplast; serial sections were cut at 7 µm thickness and stained with Masson's trichrome.

For the studies of the embryonic development of *Buccinum undatum* Linnaeus, 1758, the egg cases were collected by SCUBA diving in the vicinity of the Biological Station of Moscow State University in Kandalaksha Bay, on the White Sea. The egg cases were maintained in the laboratory in a running seawater aquarium. Capsules were dissected periodically, and embryos preserved in phosphate-buffered 2.5% glutaraldehyde (pH 7.6).

Fixed embryos were dehydrated in graded ethanol series and embedded in epone-araldite medium. Sections were cut at 2–2.5 µm thickness and were stained with methylene blue and toluidine blue in borax. Sections were examined using a Carl Zeiss Axioplan 2 microscope and photographed with an Axio-Cam digital camera.

RESULTS

Nassariidae

Nassarius luteostoma Broderip and Sowerby, 1829
(Figure 1)

Material Examined: Two specimens sectioned, Panama, Pacific Ocean: Venado Island, at low tide on sandy bar, 08°52'48.6" N, 79°35'36.9" W, coll. Yu. Kantor, 2006. The valve of Leiblein is large, pear-shaped, about twice as broad as the anterior esophagus, situated immediately in front of the circumoesophageal nerve ring. Its histology is very similar to that described by Brown (1969) for *Ilyanassa obsoleta*. The cone-shaped papilla (Figure 1, **csp**) is lined by columnar ciliated epithelium which is continuous with that of the anterior esophagus. The cells on the top of the papilla bear extremely long cilia of about 400 µm in length and that span most of the valve length. At the base of the papilla there is a ring of tall, ciliated, light-staining cells confluent with the papilla. In longitudinal section, this ring of cells looks like a triangle (Figure 1, **lsc**). This ring is usually called a mucous pad (Fretter and Graham, 1962; Andrews and Thorogood, 2005), and it is thought that its main function is to produce mucus that binds the particles. This ring of cells is seen as a whitish circle through the valve walls.

The thickened part of the valve is composed of pseudostratified columnar ciliated epithelium (Figure 1, **pse**). The cells are stained dark blue. No traces of the dorsal folds of the anterior esophagus were found.

The outer surface of the valve has an extremely thin layer of muscle fibers (in contrast to the relatively thick layers of longitudinal and circular muscles that form the wall of the anterior esophagus) and a rather thick layer of connective tissue (Figure 1, **ct**).

Buccinidae

Triumphis distorta (Wood, 1828)
(Figure 2)

Material Examined: Two specimens sectioned, Panama, Pacific Ocean: Playa Bique, in rock crevices, high in intertidal zone, 08°52'42.3" N, 79°39'18.8" W, coll. Yu. Kantor, 2006.

The valve is large, subcylindrical, about 1.5 times as broad as the anterior esophagus, situated at some distance in front of the nerve ring. Histology of the valve is rather similar to that of *N. luteostoma*, although due to the fixation conditions it seems slightly distorted. The cilia of the cells of the cone-shaped papilla reach at least 1100 μm in length. The ring of the light-staining cells confluent with the papilla is less pronounced (Figure 2, **lsc**). The pseudostratified epithelium lining the valve forms two different zones. The anterior zone, rather narrow and adjoining the cone-shaped papilla is stained very dark blue (Figure 2, **pse**) and similar in histology and staining properties to that of *Nassarius luteostoma*. This type of epithelium is sharply replaced by light staining columnar epithelium, composed of two types of cells. The first one extends from basement membrane to the lumen (Figure 2, **lpse**), bears cilia, and has small nuclei that are located close to the apical tip. The second type of cells extends to approximately 2/3 the height of the tissue layer, and does not reach the lumen. Their nuclei are situated in the basal 1/3 of the cytoplasm. This type of epithelium occupies a much longer zone of the valve and adjoining part of the mid-esophagus, so that in total it is three times as long as the expanded part of the valve proper.

The outer surface of the valve has an extremely thin layer of muscle fibers and no connective tissue.

Muricidae

Muricanthus radix (Gmelin 1798)
(Figures 3–5)

Material Examined: Two specimens sectioned, Panama, Pacific Ocean: Venado Island, on rocks at low tide, 08°52'48.6" N, 79°35'36.9" W, coll. Yu. Kantor, 2006.

The valve is large, pear-shaped, 3.5 times as broad as the anterior esophagus, situated immediately in front of the nerve ring. The columnar ciliated epithelium, lining the anterior esophagus, is sharply replaced by very tall columnar epithelium at the entrance to the valve. These tall epithelial cells form the large cone-shaped papilla with broad lumen. The cells on the top and external wall of the papilla bear long cilia (Figure 5, **cil**), around 750 μm in length. The mucous pad at the base of papilla is absent. The thickened part of the valve is composed of tall, columnar, folded ciliated epithelium (Figure 3, **cle**). Due to the staining properties, the nuclei were not seen.

The location of torsion is seen from the exterior, lies in the middle of the valve. The dorsal groove of the anterior esophagus interrupts the cone-shaped papilla and can be traced along the entire valve length (Figure 4, **dg**).

The outer surface of the valve has a very thin layer of muscle fibers (in contrast to the relatively thick layers of longitudinal and circular muscles that form the wall of the anterior esophagus) and no connective tissue.

Conidae, Raphitominae

Paramontana rufozonata (Angas, 1877)
(Figures 6–8)

Material Examined: One specimen sectioned, Western Australia, Rottnest Island, Cape Vlamingh, intertidal rocks, coll. J.D. Taylor, 1996.

The valve of Leiblein is very small, funnel-shaped, situated immediately posterior to the buccal mass and in front of the nerve ring. It is about twice as broad as the esophagus. The wall of the valve consists of a single layer of ciliated epithelial cells, slightly taller cells form the cone-shaped papilla. These cells bear long cilia (around 120 μm in length). No other structures can be recognized within the valve.

Cancellariidae

Plesiotriton vivus Habe and Okutani, 1981
(Figures 9–10)

Material Examined: Two specimens sectioned, Philippines, Bohol/Sulu seas, R/V DA-BFAR, PANGLAO 2005 Deep-Sea Cruise, st. CP 2359, 8°49.9' N, 123°34.9' E, 437–476 m.

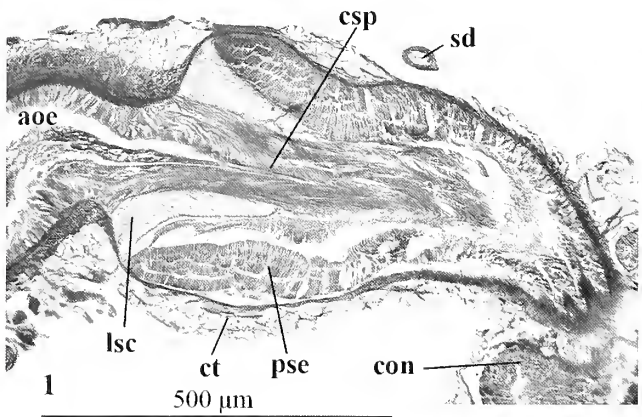
The enlargement of the esophagus (Figure 9, **vl**), which was recognized as the valve of Leiblein by Graham (1966), lies immediately posterior to the buccal mass in the anterior part of the extremely long, coiled proboscis, and is partially covered by the tubular salivary glands. The structure is coiled, forming at least two complete whorls, meaning that this is not a site of torsion (where the rotation of the esophagus would not exceed 180°). Through the semi-transparent walls of the valve, the narrow strip of opaque white tissue running along the entire length of the valve is clearly seen. On external view, it looks similar to the ring of tall, ciliated light-staining cells (= the mucous pad) in the valve of other neogastropods.

In histological sections, this strip is represented by light-staining, low, non-ciliated, large epithelial cells with large, oval nuclei. The remaining wall of the valve is lined with very tall pseudostratified ciliated epithelium, composed of two cells types. Cells of the first type extend from the basement membrane to the lumen, bear cilia, and have small, narrow, elongated nuclei that are located in the upper 1/3 of the cytoplasm. Cells of the second type do not reach the lumen and have rounded nuclei that are situated in the basal most part of the cytoplasm. The cone-shaped papilla is absent. The dorsal groove and folds were clearly seen within the valve. The relatively broad lumen of the valve was filled with blue-staining secretion.

The outer surface of the valve has a very thin layer of muscle fibers (in contrast to the relatively thick layers of longitudinal and circular muscles that form the wall of the anterior esophagus) and hardly any connective tissue.

Olividae

Oliva bulbosa (Röding, 1798)
(Figures 11–12)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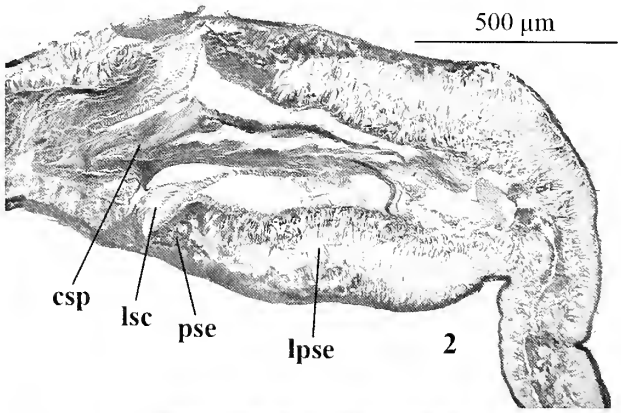
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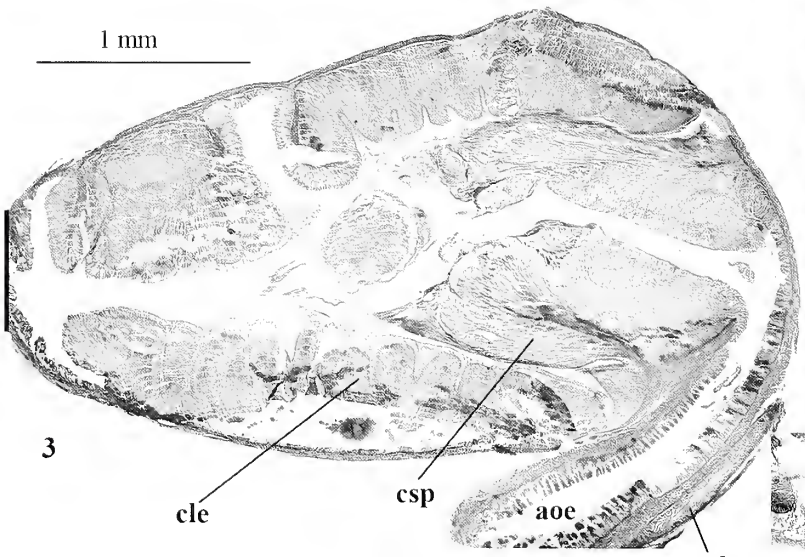
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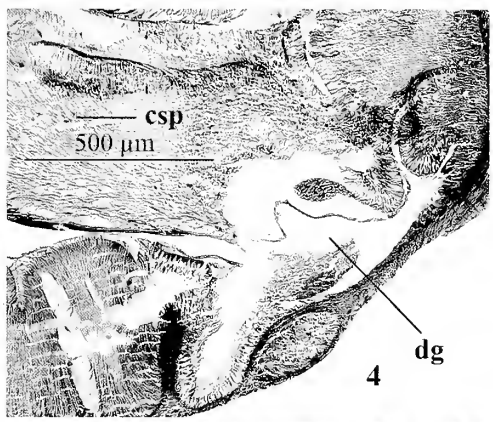
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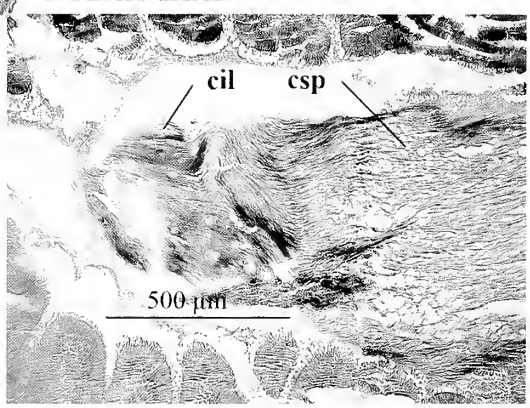
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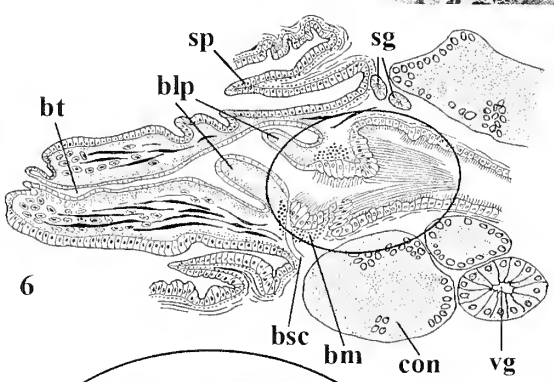
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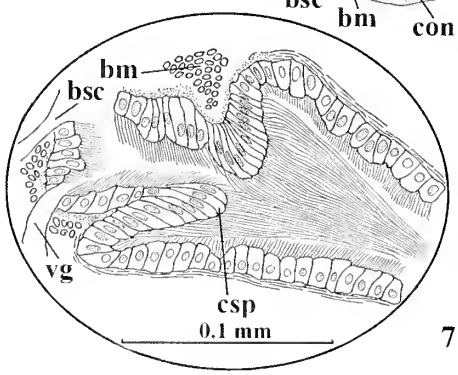
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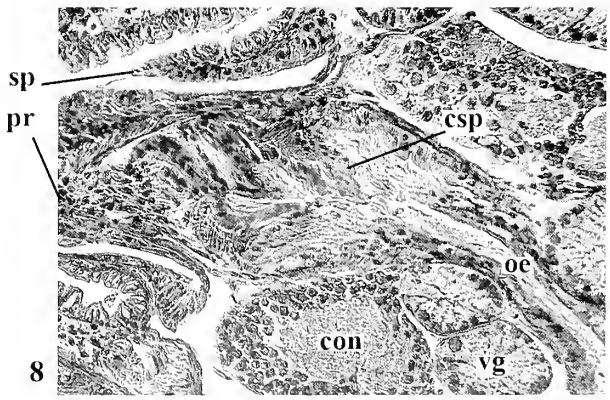
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Material Examined: One specimen sectioned, Aden Bay, sandy beach 6 km west of Aden; Red Sea, coll. D. Ivanov.

The valve is large, at least 3.5 times as broad as the esophagus, pear-shaped, and situated immediately in front of the nerve ring. The cone-shaped papilla (Figure 11, **csp**) is lined by columnar ciliated epithelium which is continuous with that of the anterior esophagus. The cells on the top of the papilla bear long cilia about 200 μm in length (Figure 12, **cil**). Probably due to the contraction of the papilla, its inner lumen was not observed. At the base of the papilla there is a ring of tall extremely light-staining cells confluent with the papilla (Figure11, **lsc**). The thickened part of the valve is composed of pseudostratified columnar ciliated epithelium (Figure 11, **pse**). The cells are stained dark blue. No traces of the dorsal folds of the anterior esophagus were found inside the valve. The outer surface of the valve has an extremely thin layer of muscle fibers.

DISCUSSION

Morphological Comparisons of the Valve of Leiblein Among Different Lineages of Neogastropoda: Graham (1941) described the significant differences in the foreguts of *Nucella* and *Buccinum*, and suggested their independent origins from different groups because they exhibit different positions of torsion in the mid-esophagus. In *Nucella*, torsion occurs within the valve, while in *Buccinum* the position of torsion is posterior to the nerve ring. Ponder (1974) did not consider the position of torsion to be of great importance, and did not dispute the homology of the valve. At the same time he pointed out the significant differences among taxa in the position of the valve relative to the buccal mass. While in most of the Neogastropoda the valve lies immediately in front of the nerve ring, in Cancellarioidea it is situated just behind the buccal cavity, with the mid-esophagus positioned in front of the nerve ring (Graham, 1966).

The data presented confirm the significant morphological variability of the "valve of Leiblein" found in different lineages of the Neogastropoda. The most-divergent "valve" from the few described in literature was found in *Plesiotriton* (Cancellariidae), in which it is coiled and forms at least two complete whorls. The cone-shaped papilla and the ciliary valve are completely absent (Figures 9–10). The way in which it functions is unclear. The position of the valve itself in the most-anterior part of the proboscis is unusual for the Neogastropoda, but it position in relation to the buccal mass is similar to that in Conoidea.

In the remaining families studied, the valve of Leiblein demonstrates a higher degree of similarity, being pear-shaped and possessing the cone-shaped papilla either formed by or lined with epithelium with very long cilia, varying from 120 μm (*Paramontana rufozonata*) to 1100 μm (*Triumphis distorta*) in length. In relation to the circumoesophageal nerve ring the valve in adults is always positioned in front of the ring. The other character common to all the studied species is that the walls of the valve lack any substantial muscle layer, unlike the walls of the adjoining part of the esophagus.

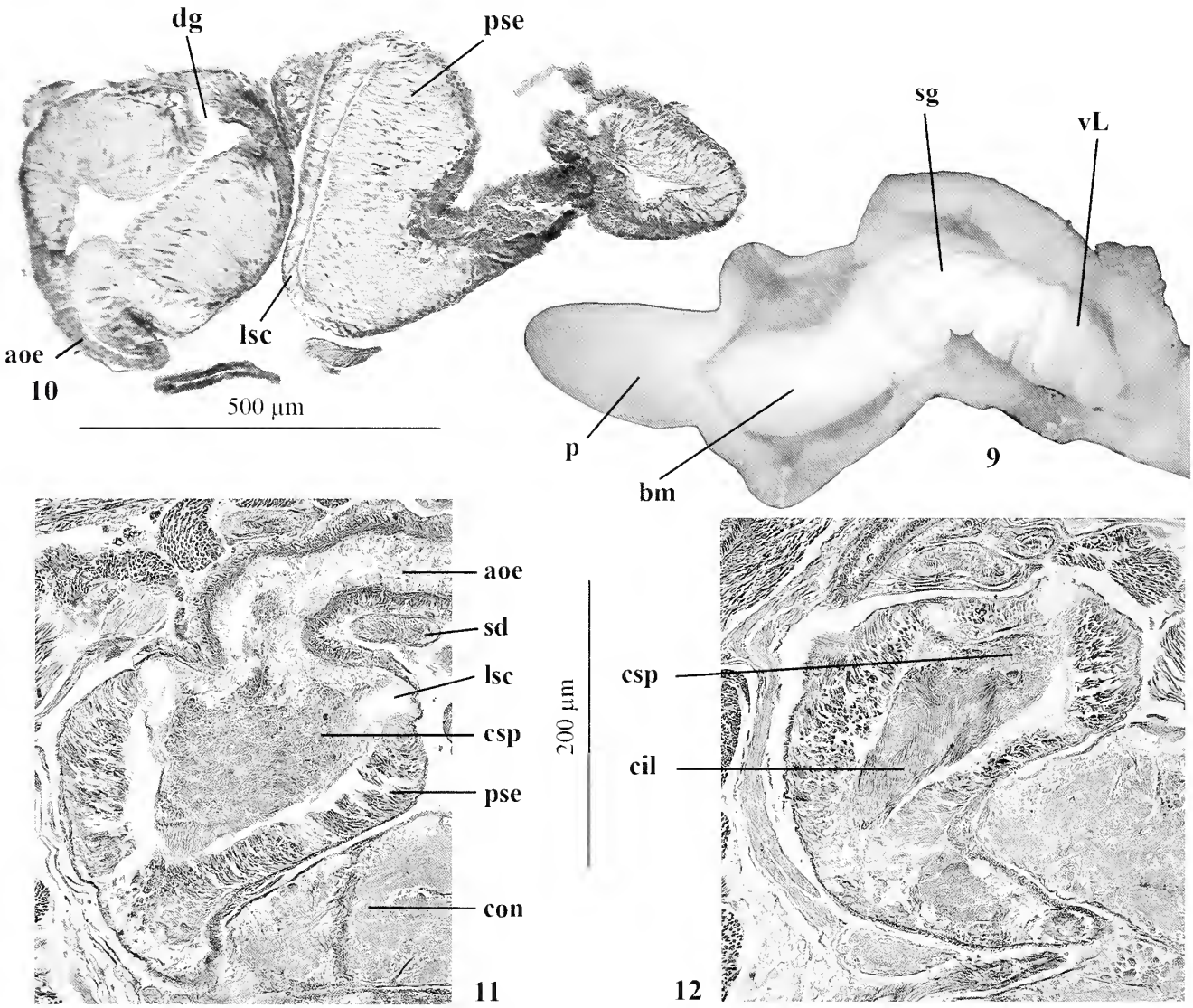
Within Conoidea presence of a valve was recorded only in two species, *Paramontana rufozonata* and *Kermia barnardi* (Brazier, 1878) (Kantor and Taylor, 2002). Both species have a valve of very similar structure, which is formed by only a single layer of cells. It should be noted that these species are characterized by a very small shell (less then 5 mm). Therefore the valve seems to be very much simplified due to the minute size of the mollusks.

Some significant differences can be found among the valves studied to date, mostly in the presence/absence of the dorsal groove of the anterior esophagus within the valve. It can be clearly observed in nearly all families studied—Muricidae, Cancellariidae, Volutidae (Ponder, 1970), Costellariidae (Ponder, 1972), and Volutomitridae (Kantor and Harasewych, 1992). It is absent in studied Buccinoidea, including Fasciolariidae (Marcus and Marcus, 1962), as well as in Olividae and Conidae (our data). Another difference among valves has to do with the position of the valve in relation to the site of the torsion. Torsion is situated posterior to the valve in all taxa except Muricidae. We were not able to trace the torsion site in Conidae due to the minute size of the animal.

Another difference observed was the presence/absence of the ring of the ciliated light-staining cells (mucous pad). It was mentioned for every studied species possessing the valve of Leiblein, but surprisingly it was absent in *Muricanthus* (although present in *Nucella*). It was similarly absent in two species of Conidae.

Development of the Valve in Ontogeny: These differences observed in the histology of the valve of Leiblein prompted us to check whether its development is identical in the embryogenesis of different neogastropod lineages.

Figures 1–8. The valve of Leiblein. **1.** *Nassarius luteostoma* Broderip and Sowerby, 1829, longitudinal section through the valve. **2.** *Triumphis distorta* (Wood, 1828), longitudinal section through the valve. **3–5.** *Muricanthus radix* (Gmelin, 1791). **3.** Longitudinal section through anterior esophagus and valve. **4.** Enlarged fragment of the longitudinal section showing the dorsal groove of the anterior esophagus interrupting the cone-shaped papilla. **5.** The tip of the cone-shaped papilla showing the long cilia. **6–8.** *Paramontana rufozonata* (Angas, 1877). **6.** Semi-diagrammatic longitudinal section through proboscis, buccal mass, and valve. **7.** Enlarged semi-diagrammatic section through the valve. **8.** Histological section through the valve. Abbreviations: **aoe,** anterior esophagus; **blp,** buccal lips; **bm**, buccal mass; **bsc,** buccal sac; **bt,** buccal tube; **cil,** cilia; **cle**, columnar folded ciliated epithelium; **con,** circumoesophageal nerve ring; **csp,** cone-shaped papilla; **ct,** connective tissue; **dg,** dorsal groove; **lpse,** light staining columnar epithelium; **lsc,** light-staining cells; **pse,** pseudostratified epithelium; **sd,** salivary duct; **sg,** salivary gland; **sp,** septum of the rhynchocoel; **vg,** venom gland.

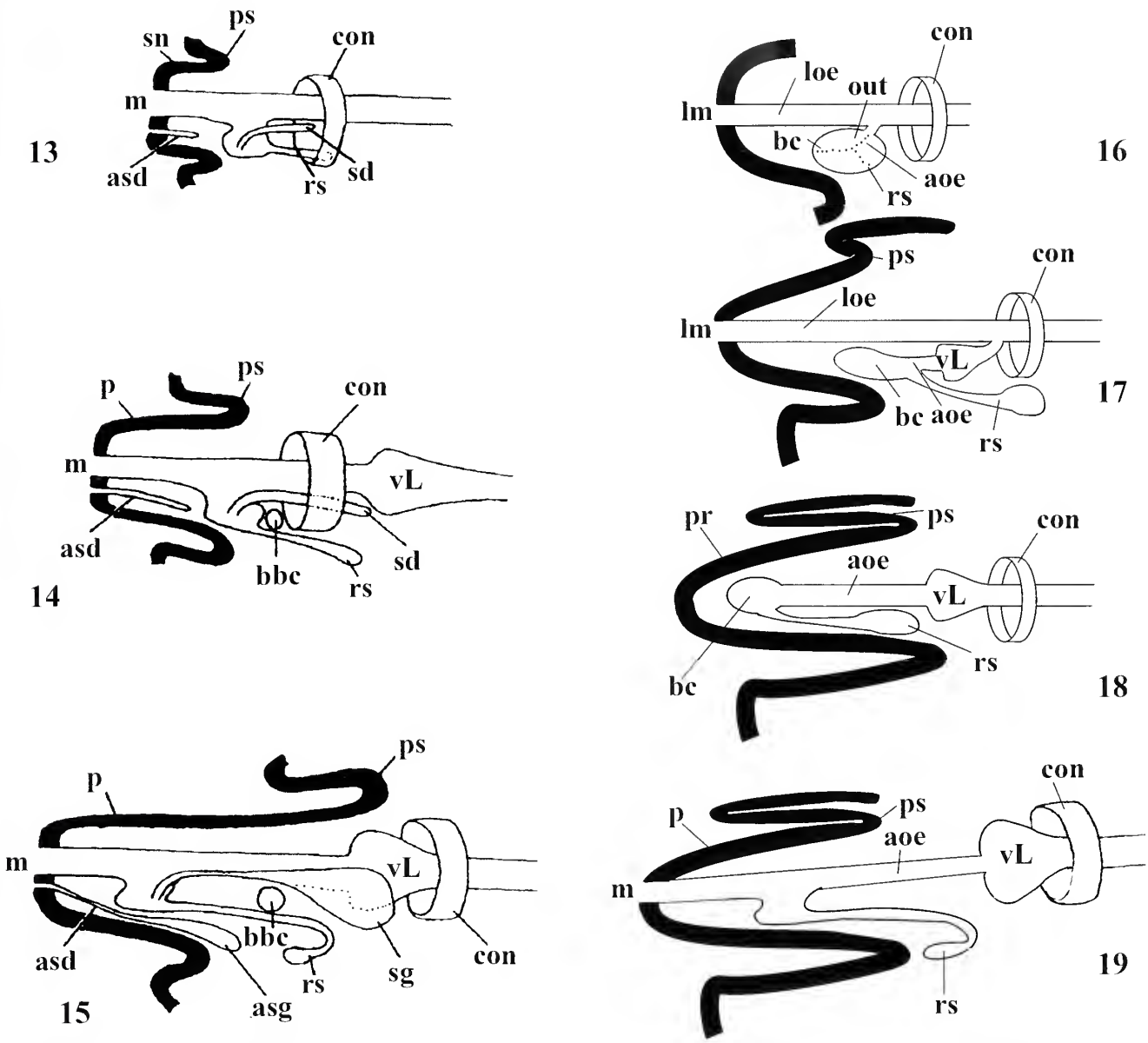


Figures 9–12. Valve of Leiblein. **9–10.** *Plesiotriton vivus* Habe et Okutani, 1981. **9.** Anterior part of the proboscis dissected to show the position of the valve in relation to the buccal mass. **10.** Longitudinal section through the anterior esophagus and the coils of the valve. **11–12.** *Oliva bulbosa* (Röding, 1798). **11.** Longitudinal section through anterior esophagus and valve. **12.** Longitudinal section through valve and mid-esophagus, showing the long cilia of the cone-shaped papilla.Abbreviations: **p,** proboscis; **vL**, valve of Leiblein. Other abbreviations see in captions to Figures 1–8.

There is very little published data on the development of the valve of Leiblein in ontogeny. Ball et al. (1997a, b) examined the ontogeny of the foregut in *Nucella lapillus* (Figures 13–15).

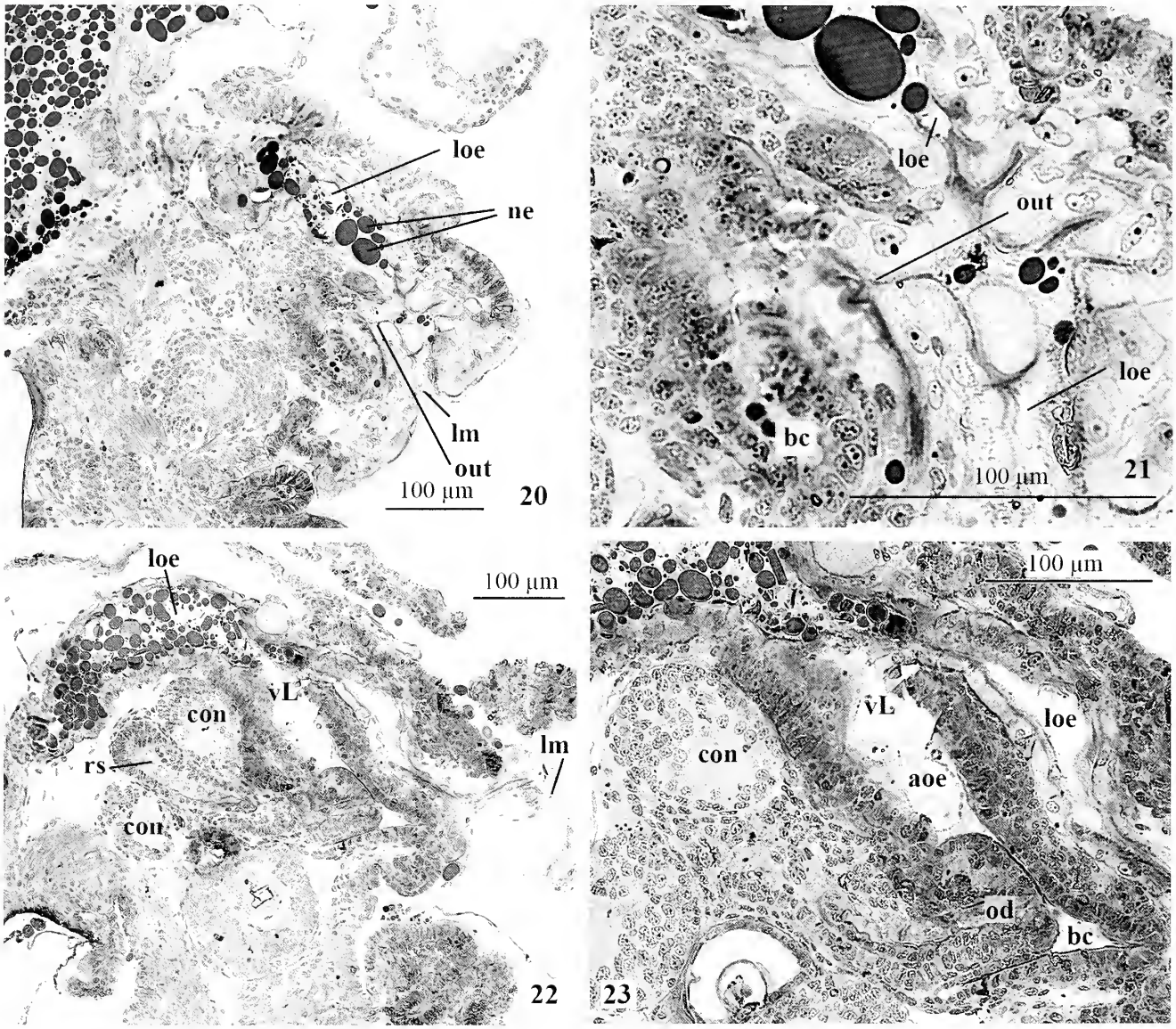
Abro (1969) (summarized by Fretter, 1969) examined the embryology of *Nassarius incrassatus* (Ström, 1768) and *N. reticulatus* (Linnaeus, 1758) (Nassariidae). Page (2005) re-examined the development of the foregut and proboscis in a different nassariid species, *Nassarius mendicus* (Gould, 1850) with planktotrophic larvae and illustrated it by a series of outstanding photographs. We complemented the data on the Nassariidae by observations of direct developing embryos of *Buccinum undatum*.

Published and original data on the embryonic development of the valve in Buccinidae and Muricidae demonstrated that it originates from different sections of the anterior foregut. From the diagrams of Ball et al. (1997a, b), it is obvious that in *Nucella*, the buccal mass with the radula originated from the ventral outpocketing of the esophagus during the early stages of proboscis formation (Figure 13). The valve of Leiblein appeared in the next stage (Figure 14), as development of the esophagus posterior to the nerve ring. Later, the progressive elongation of the proboscis pulls the salivary glands, radular sac, and the valve through the nerve ring into their final positions (Figure 15). Thus, the valve is formed as part of the anterior larval esophagus.



Figures 13–19. Diagrammatic lateral view of the development of the foregut and proboscis. **13–15.** *Nucella lapillus* (after Ball et al., 1997a, b, modified). **13.** Stage 6, the buccal mass has developed. **14.** Stage 7, the valve of Leiblein lies posterior to the cerebral commissure. **15.** Stage 8, the valve of Leiblein, acinous salivary glands, and the radular sac lie anterior to the nerve ring. **16–19.** Selected stages of the foregut development in Buccinoidea (based on Page, 2005, on *Nassarius mendicus* (Gould, 1850) and observations on *Buccinum undatum*). Salivary glands are omitted for simplicity. **16.** Formation of the ventral outpocketing. **17.** Formation of buccal cavity, anterior esophagus, valve of Leiblein, and radular sac. Larval esophagus still open. **18.** Larval esophagus resorbed, larval mouth opening is sealed. **19.** Postmetamorphic new mouth is formed. Abbreviations: **asd,** duct of accessory salivary gland; **asg,** accessory salivary gland; **bbc**, buccal commissure; **bc**, buccal cavity; **lm,** larval mouth; **loe**, larval esophagus; **m**, mouth; **out**, outpocketing; **ps,** proboscis sheath; **sn,** snout. Other abbreviations as in captions to Figures 1–12.

The situation with Nassariidae and *Buccinum* differs significantly. In these groups, the larval esophagus is initially a ciliated tube that extends from the mouth to the stomach. A patch of enlarged, non-ciliated cells is embedded within the ventral wall of the distal larval esophagus and forms an outpocketing (Figure 16, 20–21). Eventually, the outpocketing will enlarge and become extensively elaborated to form the entire post-metamorphic foregut. At first, the future buccal cavity, radular sac, and valve of Leiblein form from different chambers of the original outpocketing (Figures 17, 22–23). With the enlargement of these structures as well as elongation of the proboscis, dramatic changes take place. Most notable among these events are the complete occlusion of the larval mouth and the degeneration and loss of the distal larval esophagus between the larval mouth and the point



Figures 20–23. Selected stages of development of *Buccinum undatum*. **20.** Mid-sagittal section through a larva. Ventral outpocketing formed. The stage corresponds to Figure 16. **21.** Enlarged region of Figure 20. **22–23.** Formation of buccal cavity, anterior esophagus, valve of Leiblein, and radular sac. Larval esophagus still open. The stage corresponding to Figure 17. **23.** Enlarged regiont of Figure 22. Abbreviations: **ne,** nurse eggs; **od,** odontophore. Other abbreviations see in captions to Figures 1–19.

where the post-metamorphic foregut extends from the ventral side of the larval esophagus (Figure 18). Later, the new definitive mouth ruptures through the transient epithelial seal that formed over the larval mouth (Figure 19) (Page, 2005, figs. 2 B, C). Thus the overall similarity of adult foreguts of Buccinoidea and Muricidae in fact is achieved through very different processes. We want to emphasize that the radular apparatus in both stems originates from homologous structures—the ventral outpocketings of the esophagus. In contrast, the "valve" originated from different parts of the foregut—from the posterior chamber of ventral outpocketing in Buccinidae and part of the anterior larval esophagus in Muricidae.

Our attempts to examine the entire development of the foregut in embryos of *Buccinum undatum* failed due to asynchronous development of the embryos even within the same egg cluster and egg capsules. Therefore it was not possible to obtain the embryos on consequent developmental stages with any reliable timing. Nevertheless, we were able to observe the early stages which roughly corresponded to approximate halfway point of obligatory larval development (21 days post-hatching) in *Nassarius mendicus* (Page, 2005).

The major difference between studied nassariids and *Buccinum* is that the nassariids are characterized by feeding planktonic larvae, while *Buccinum* has direct development, feeding on nurse eggs inside the egg capsule and

hatching in the crawling stage. The nurse eggs are consumed in rather early stages, and are clearly seen in the larval esophagus (Figures 20–2, **ne**). We have not observed the stage with the degenerated larval esophagus, but have seen an example of the strongly differentiated initial outpocketing giving rise to the radular sac, in which the radular teeth were seen, and the buccal mass with odontophore and future anterior esophagus with valve of Leiblein was situated in exactly the same position as in *N. mendicus* (Figures 22–23). Therefore, it is presumed that the development of the valve in *Buccinum undatum* is analogous to that in *Nassarius*.

It should be emphasized that the development of the valve seems to be unrelated to the mode of embryogenesis. Similar developmental patterns were found in related species with planktotrophic (*Nassarius*) and lecitotrophic larvae (*Buccinum*), while unrelated species with lecitotrophic larvae (*Buccinum* and *Nucella*) differed in the development of the valve. Both *Buccinum* and *Nucella* feed on the nurse eggs during the first stages of the development (Fretter and Graham, 1962).

Our preliminary data demonstrated significant differences in the morphology of the valve of Leiblein in different groupings of Neogastropoda, and different origins of the valve during embryogenesis, at least in Muricidae and Buccinidae. This suggests that, despite the superficial similarity, the homology of the valve of Leiblein within Neogastropoda is at best questionable.

If this supposition is correct, then Buccinoidea do not share any of the previously hypothesized autapomorphies with the rest of neogastropods. This raises the prospect of a paraphyletic Neogastropoda that includes two stems, one including the Buccinoidea, the other containing the remaining neogastropod families.

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Phylogenetic analysis of the subfamily Colinae (Neogastropoda: Buccinidae) based on morphological characters

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ABSTRACT

Colinae Gray, 1857, the most abundant and diverse subfamily of whelks in the northwestern Pacific and Far-Eastern Seas of Russia, includes several conchologically similar genera or subgenera of unclear status and composition. Based on morphological and anatomical studies of 38 species attributed to the genera *Colus* Röding, 1799, *Plicifusus* Dall, 1902, *Latisipho* Dall, 1916, *Aulacofusus* Dall, 1918, *Retifusus* Dall, 1916, *Retimolnia* McLean, 1995, and *Pararetifusus* Kosuge, 1967, a partial generic revision and phylogenetic analysis based on 34 characters is produced. The resulting majority rule consensus tree well resolves the genera *Plicifusus*, *Retifusus*, *Pararetifusus*, and *Aulacofusus*. The genus *Retimolnia* appears to be a junior synonym of the genus *Retifusus*. Species of the heterogeneous genus *Colus* included in this study do not form a clade, indicating that this genus, as presently understood, is paraphyletic. Our results demonstrate the importance and utility of anatomical characters for resolving the systematics of the extremely diverse and variable family Buccinidae.

Additional keywords: Taxonomy, phylogeny, cladistics, northwestern Pacific

INTRODUCTION

Although the number of papers dedicated to the molecular phylogeny of neogastropods continues to increase, there is no parallel inerease in data on their morphology and anatomy. This is especially true for the Buccinidae, a large and evolutionarily suecessful family of predatory marine gastropods that are widespread in polar, temperate, and tropical waters of the World Ocean, and which have signifieant commereial value. In the northwestern Pacific, Buceinidae is one of the dominant families, and in waters of the Russian Far-East, it is the most abundant and diverse family, comprising more than 30% of the total number of gastropod species (Kantor and Sysoev, 2006). Six bueeinid subfamilies are present in the northwestern Pacifie: Buccininae Rafinesque, 1815; Colinae Gray, 1857; Beringiinae Golikov and Starobogatov, 1975; Ancistrolepidinae Habe and Sato, 1973; Parancistrolepidinae Habe, 1972; and Volutopsiinae Habe and Sato, 1973. The subfamily Colinae (previously better known under the name Neptuneinae Stimpson, 1865) is the most diverse with respect to the number of genera and species in the northwestern Pacific (Kantor and Sysoev, 2005, 2006). It includes 16 of the 34 genera and 116 of the 263 species of Buecinidae reeorded in the fauna of Russia.

The best known representative of this subfamily is the diverse genus *Neptunea*, whieh has had two recent revisions (Golikov, 1963; Fraussen and Terryn, 2007). Other genera, with species that do not grow to commercial size, have not attracted sufficient attention of malacologists. Among them are several conchologically similar genera with unclear taxonomic status and species composition, ineluding: *Colus* Röding, 1799, *Latisipho* Dall, 1916, *Plicifusus* Dall, 1902, *Aulacofusus* Dall, 1918, *Retifusus* Dall, 1916, *Pararetifusus* Kosuge, 1967, and *Retimolnia* McLean, 1995.

Species and genera within Buccinidae have generally been diagnosed based primarily on conchological characters, with radular morphology contributing only oecasionally to their taxonomy. Anatomical characters have, thus far, hardly been used for these purposes.

The aim of this publication is to clarify the status and composition of the genera *Colus* Röding, 1799, *Plicifusus* Dall, 1902, *Latisipho* Dall, 1916, *Aulacofusus* Dall, 1918, *Retifusus* Dall, 1916, *Retimolnia* McLean, 1995, and *Pararetifusus* Kosuge, 1967, based on conchological, anatomical and radular characters, as well as to evaluate the utility of morphological characters for resolving the taxonomy of Colinae.

MATERIALS AND METHODS

We dissected and analyzed the anatomy of 38 species of Colinae, defining 34 characters coded as 82 character states that were nsed to perform the phylogenetic analyses of these taxa (Table 1, Appendix 1). Of these, 7 eharaeters described shell structure, 5 characters the

soft body and the mantle, 5 characters the reproductive systems, 12 characters the digestive system, and 5 characters the structure of the radula. The material for the study was obtained from the Zoological Institute (Saint Petersburg, Russia), the P. P. Shirshov Institute of Oceanology of Russian Academy of Sciences (Moscow), and the Zoological Museum of Moscow State University. In total, nearly 200 specimens were dissected. While processing this material, standard zoological methods were used, such as manual dissection, histology and scanning electron microscopy for the examination of radulae. Phylogenetic analyses were run using Paup*4 (Swofford, 1998).

RESULTS

Brief Descriptions of the Taxonomically Informative Morphological Characters of the Studied Genera: The gross anatomy of Colinae is typical of the Buccinidae in general features (Figures 1–2). The operculum may have a terminal (*Latisipho, Colus, Aulacofusus*; Figures 1, 26), or subspiral nucleus (*Pararetifusus*; Figure 4). The mantle cavity spans approximately one whorl of the body (Figure 3). The ctenidium (**ct**), osphradium (**os**) and, in females, the capsule gland (**cg**) can be observed by partial transparency of the mantle. Relative sizes of the ctenidium and osphradium vary in different species.

Penis morphology was used successfully by Golikov (1963, 1980) for taxonomic studies of the genera *Neptunea* and *Buccinum*; however, in our study, the structure of the distal section of penis varied very little. In *Latisipho, Plicifusus,* two species of *Colus*, and several *Retifusus* species, the seminal duct opens at the tip of a large, cone-shaped papilla (Figures 6, 8–9, **sp**) that is encircled by a fold of skin (**cf**). In the remaining *Retifusus* species, the seminal papilla is very small and becomes narrower towards its tip (Figure 10, **sp**). In the genus *Pararetifusus*, the seminal papilla is absent, with the male orifice situated terminally at the tapering tip of the penis (Figure 5). The structure of the pallial gonoduct in females appeared to be even more conservative in the genera studied. In the majority of species we examined, the vagina is strongly developed (Figure 11), occupying a ventral position on the capsule gland. Only in the genus *Pararetifusus* it is situated terminally.

The mouth opening is situated at the tip of a more or less elongated proboscis (Figure 12, **mo**). While contracted, the proboscis is situated within the rhynchodeum (Figure 12, **rd**). The anterior section of the rhynchodeum is immovable and attached to the body haemocoel walls by multiple tensor muscles. The posterior section of the rhynchodeum is capable of being everted. The proboscis is retracted by retractor muscles attached to the rhynchodeum walls (Figure 12–15, **prr**). The longest proboscises in the contracted state were found in *Aulacofusus* and in some species of *Colus*, where they are folded within the rhynchodeum (Figure 14). In other genera (*Plicifusus, Retifusus,* and *Latisipho*), the proboscis remains straight within the rhynchodeum (Figures 12–13), and elongates mostly due to eversion of the posterior, movable section.

The proboscis wall is formed of an epithelium, one or two layers of circular muscle fibers, and two layers of longitudinal muscle fibers. The sequence of layers in the majority of studied genera (*Aulacofusus*, *Latisipho*, *Retifusus*) is, (from outer to inner surfaces): epithelium, circular muscle layer, longitudinal muscle layer, circular muscle layer, and an innermost longitudinal muscle layer (Figures 18–20). In two studied species of *Plicifusus*, the sequence of layers differed, consisting of: epithelium, longitudinal muscle layer, circular muscle layer, and longitudinal muscle layer in *P. lyastarius* (Figure 24), with the addition of an innermost, second layer of circular muscle fibers in *P. rhyssus*.

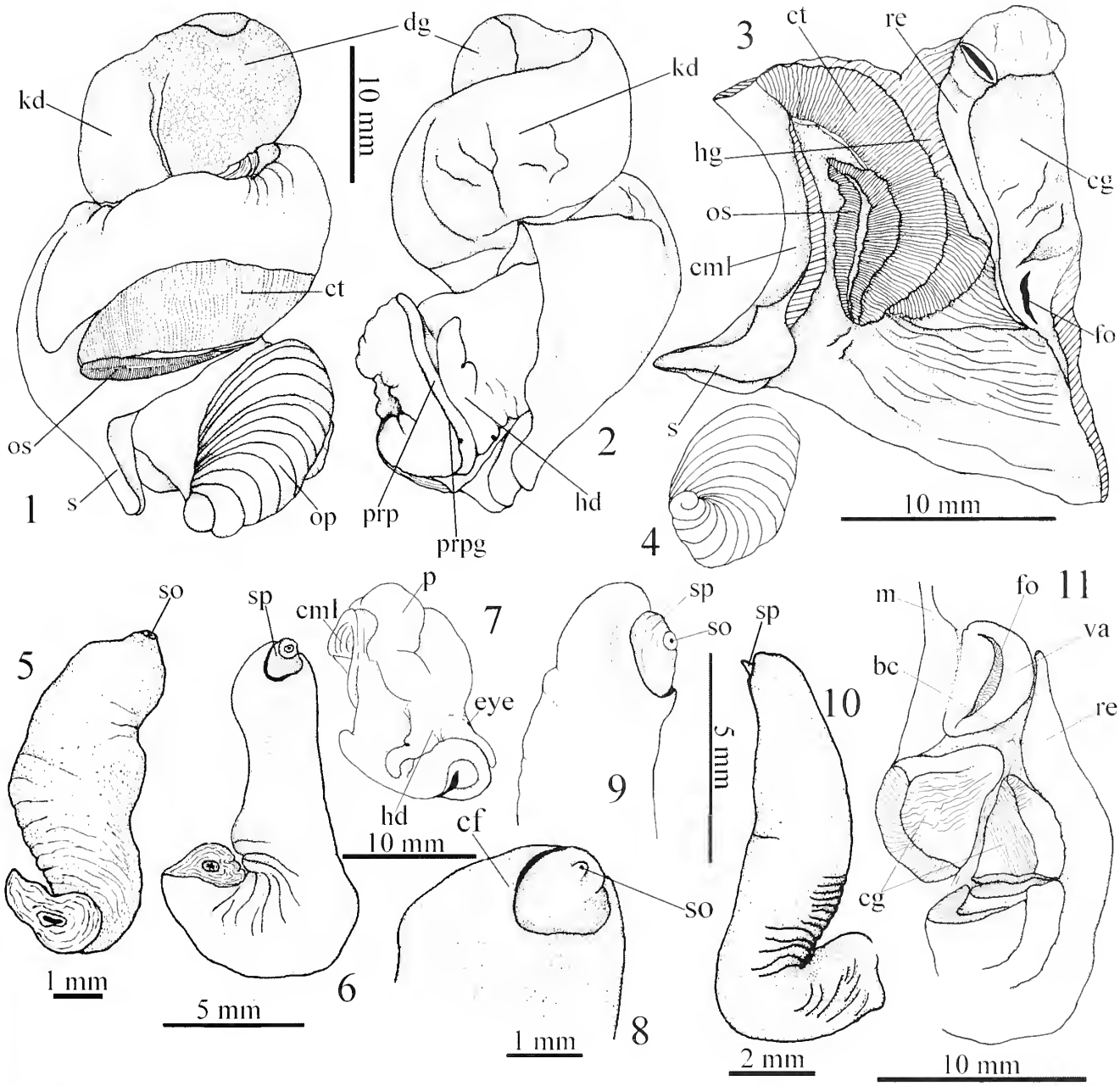
Within the proboscis is the buccal mass with radula. Comparative lengths of the buccal mass varied among taxa and have taxonomic significance. Each row of the radula (Figures 36–41) consists of two lateral teeth and one central tooth, each normally bearing 3 cusps. Although the teeth are similar in shape, the finer details are specific for genera (see below in the discussion).

The anterior esophagus opens into a large (*Retifusus, Pararetifusus*) or medium-sized (*Latisipho, Plicifusus, Colus, Aulacofusus*) valve of Leiblein (Figure 12, **vl**). The gland of Leiblein is present in all studied species (Figures 12–13, **gl**). Salivary glands differ in shape and in size (Figures 12–14, **sg**), being largest in *Retifusus* and *Aulacofusus*. The salivary ducts leave the inner side of each gland and run along the esophagus to their openings into the posterior part of the buccal cavity. The diameter and the structure of the wall of the ducts vary among different genera. In *Latisipho*, *Plicifusus*, and *Colus*, the ducts are thin and coiled (Figure 13, **sd**), while in *Aulacofusus*, *Retifusus*, and *Pararetifusus*, they are thick, sometimes with swellings in a form of a sac (salivary sacs) (Figure 15, **ss**). In *Aulacofusus*, the walls of salivary ducts have an additional layer of longitudinal nucles (Figure 21, **lm**). The posterior esophagus opens into the stomach. The structure of the stomach is generally of the same type in the majority of the species studied, but the length of the posterior mixing area can differ among genera (Figure 16–17, **pma**).

Phylogenetic Analyses: *Volutopsius norvegicus* (Gmelin, 1791) (Buccinidae: Volutopsiinae) and *Ancistrolepis okhotensis* Dall, 1925 (Buccinidae: Ancistrolepidinae), whose anatomy is known (Kantor, 1982, 1988), were used as outgroups. A heuristic search yielded 2624 trees, each 147 steps in length. Consistency index (CI) = 0.3197, homoplasy index (HI) = 0.6803, retention index (RI) = 0.6942. Figure 25 shows the 50% majority-rule consensus tree.

Several clades can be distinguished within the ingroup (Clades 1 to 6, Figure 25).

Clade 1, which is supported in 93 percent of trees, corresponds to the genus *Plicifusus*, and contains 12 species, including the type species of *Plicifusus*. At the moment, we prefer to treat it as a monophyletic genus pending examinations of additional species.



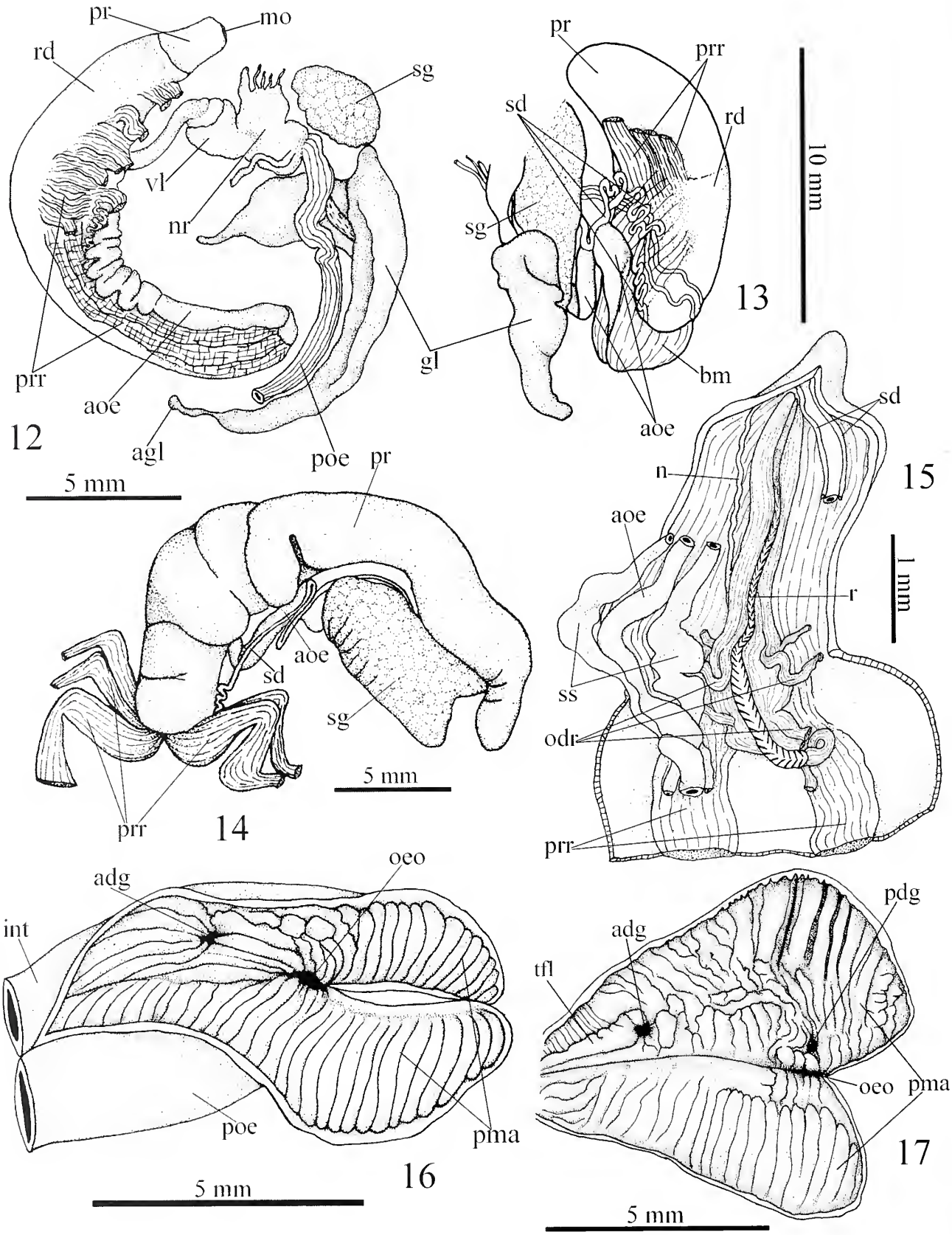
Figures 1–11. Anatomy. **1–2.** *Plicifusus bambusus*. **3.** Mantle of *Plicifusus hastarius*. **4.** Operculum of *Pararetifusus kantori*. 5. Penis of *Pararetifusus kantori*. **6, 8.** Penis of *Latisipho hallii*, ventral view. **7.** Frontal-dorsal view of the soft body of *Colus minor*, with mantle removed. **9.** Upper section of penis of *Colus minor*. **10.** Penis of *Rctifusus jessocusis*. **11.** Pallial female reproductive system of *Plicifusus rhyssus*, capsule gland, opened dorsally. Abbreviations: **bc,** bursa copulatrix; **cf,** circular fold of skin around the seminal papilla, **cg,** capsule gland; **cml,** columellar muscle; **ct,** ctenidium; **dg,** digestive gland; **eye,** eye; **fo,** female orifice; **hd,** head; **bg,** hypobranchial gland; **kd,** kidney; **m,** mantle edge; **op,** operculum; **os,** osphradium; **p,** penis; **prp,** propodium; **prpg,** propodial groove; **re,** rectum; **s,** siphon; **so,** male orifice; **sp,** seminal papilla; **va,** vagina

Plicifusus Dall, 1902

Tritonofusus (Plicifusus) Dall, 1902: 523.

Type Species: *Fusus kroyeri* Möller, 1842, by original designation.

Diagnosis: The genus is characterized by an elongated, small to medium-sized fusiform shell with well-developed axial ribs and numerous spiral cords (from 30 to 60 cords on penultimate whorl) that cover the entire shell surface (Figures 31, 33). The central tooth of the radula is large and broad, and has two to four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(usually three) sharp cusps (Figure 36). The lateral teeth usually have three or four cusps, with the central cusps always smaller than the lateral ones. The salivary ducts are very thin and convoluted. The stomach is large, as compared to the proboscis, and narrow, with a small posterior mixing area.

Remarks: *Plicifusus* was described by Dall (1902) as subgenus of *Tritonofusus* Mörch, 1857, which is an objective synonym of *Colus* Röding, 1799, since it is based on the same type species. *Plicifusus* has been treated as a distinct genus by the majority of subsequent authors.

Genus Composition: The majority of the included species were described within this genus [or attributed to the subgenus *Tritonofusus (Plicifusus)*]. *Quasisipho torquatus* Petrov, 1982, is the type species of the monotypic genus *Quasisipho* Petrov, 1982, from the upper Pliocene-lower Pleistocene of eastern Kamchatka. This species survives in the Recent fauna, and its anatomy confirms that the type species belongs within *Plicifusus*. Thus *Quasisipho* becomes junior subjective synonym of *Plicifusus*. Some species were originally described or attributed to *Retifusus* [e.g., *Plicifusus (Retifusus) scissuratus* Dall, 1918]. *Tritonofusus (Plicifusus) rhyssus* Dall, 1907 was placed in the genus *Helicofusus* Dall, 1916 (type species by original designation *Tritonofusus (Plicifusus) aurantius var. laticordatus* Dall, 1907) by many Russian authors (e.g., Kantor and Sysoev, 2005, 2006).

The results of our study place the following species within the genus *Plicifusus*:

Plicifusus kroeyeri (Møller, 1842) [= *Fusus arcticus* Philippi, 1850]
Plicifusus plicatus (A. Adams, 1863)
Plicifusus scissuratus (Dall, 1918)
Plicifusus croceus (Dall, 1907)
Plicifusus elaeodes (Dall, 1907)
Plicifusus rhyssus (Dall, 1907) [= *Plicifusus (Latifusus) wakasanus* Dall, 1918; *Tritonofusus (Plicifusus) aurantius* Dall, 1907; *Plicifusus (Aulacofusus) rhyssoides* Dall, 1918]
Plicifusus hastarius Tiba, 1980
Plicifusus bambusus Tiba, 1980
Plicifusus obtusatus Golikov *in* Golikov and Scarlato, 1985
Plicifusus olivaceus (Aurivillius, 1885) [= *Plicifusus (Retifusus) incisus* Dall, 1919]
Plicifusus oceanodromae (Dall, 1919)
Plicifusus torquatus (Petrov, 1982)

A second, well defined clade with 100% bootstrap support includes 20 species in our study, and is composed of several well supported subclades (clades 2, 3, 4, 5) and two unresolved species.

Clade 2, although not supported in all trees, contains three northern Atlantic species of the genus *Colus* Röding, 1798 (Figure 26), including *C. islandicus*, the type species. The other two species, often attributed to *Colus: C. minor* (Dall, 1925) and *C. kujianus* Tiba, 1973, do not emerge as members of this clade. These results reflect the high heterogeneity of *Colus*, which is widely distributed in the Atlantic and Arctic Oceans and in the northern Pacific. Many more species need to be studied in detail before the taxonomy of *Colus* is clearly understood.

Clade 3 includes three species belonging to the genus *Pararetifusus*, including its type species.

Pararetifusus Kosuge, 1967

Retifusus (Pararetifusus) Kosuge, 1967: 62.

Type Species: *"Phymorhynchus?" tenuis* Okutani, 1966 (by original designation).

Diagnosis: The genus is characterized by a small shell with a relatively high last whorl. The spiral sculpture consists of a few elevated, sharp or rounded ribs; axial folds are absent (Figures 28, 30). The radula is similar to that of *Retifusus roseus*, *R. laticingulatus*, *R. similis*, *R. iturupus*, and *R. attenuatus* (Figure 38) (see below for description).

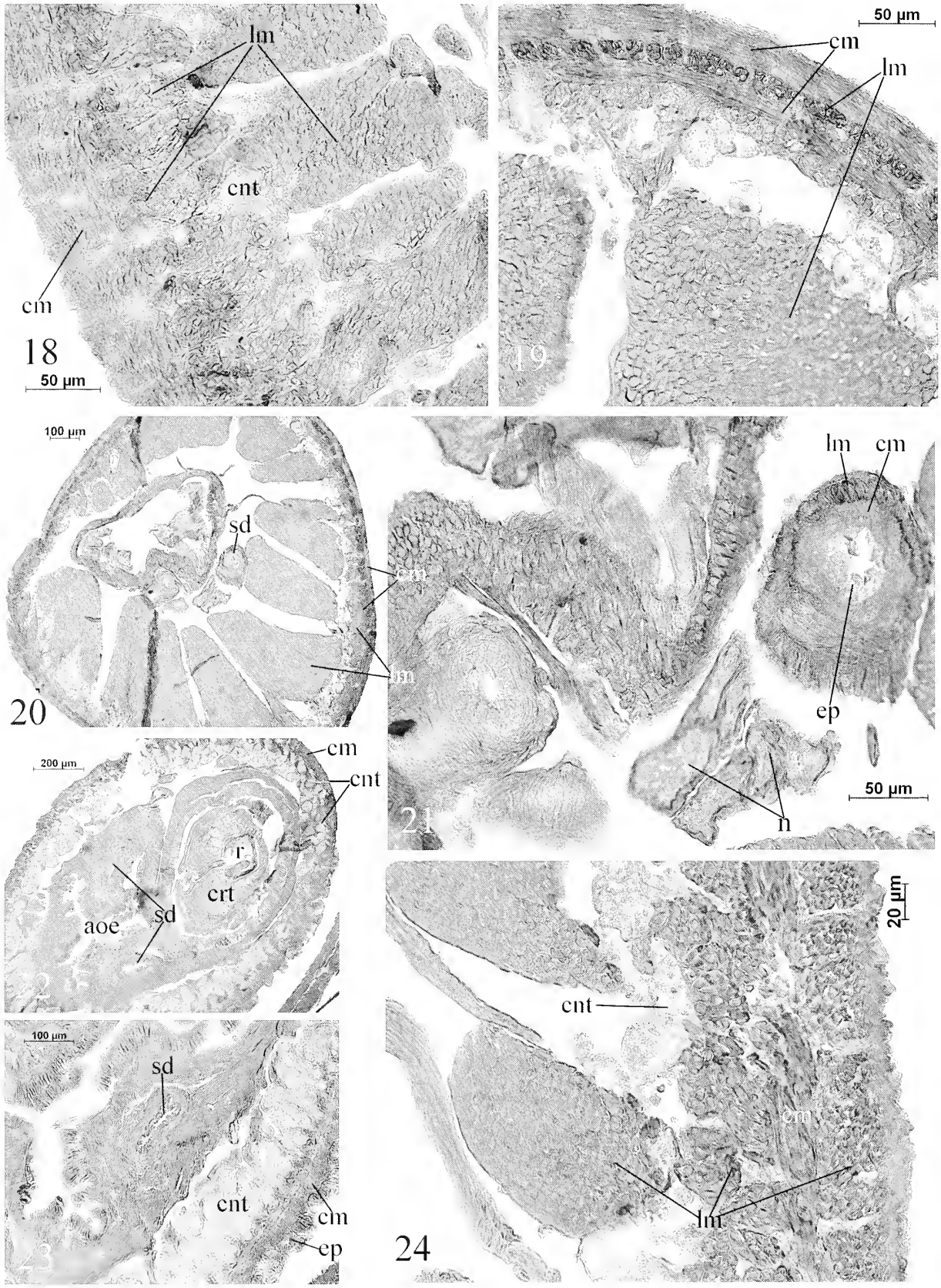
Remarks: The type species was originally placed in *Phymorhynchus* (Conoidea), but examination of the radular and morphological characters undoubtedly placed it within Buccinidae (Kosuge, 1967).

Genus Composition: Very few species have been placed in *Pararetifusus*. In addition to the species studied here (below) only one, *P. dedonderi* Fraussen and Hadorn, 2001, from Philippines was tentatively attributed to *Pararetifusus* but later excluded by Kosyan (2006a).

Pararetifusus tenuis (Okutani, 1966)
Pararetifusus kantori Kosyan, 2006
Pararetifusus kosugei Kosyan, 2006

The genus was proposed as a subgenus of *Retifusus* and is close to it in radular structure and anatomy, but differs in shell sculpture. The spiral cords of *Pararetifusus* shells are very similar to the cords of *Aulacofusus*

Figures 12–17. Anatomy. **12.** Right lateral view of the foregut of *Plicifusus hastarius*. **13.** Left lateral view of the foregut of *Plicifusus rhyssus*. **14.** Right lateral view of the foregut of *Aulacofusus herendeeni*. **15.** Dorsally opened proboscis of *Retifusus roseus*. **16.** Opened stomach of *Aulacofusus periscelidus*. **17.** Opened stomach of *Plicifusus hastarius*. Abbreviations: **adg,** opening of anterior duct of digestive gland; **agl,** ampulla of gland of Leiblein; **aoe,** anterior esophagus; **bm,** buccal mass; **gl,** gland of Leiblein; **int,** intestine; **mo,** mouth opening; **n,** nerves; **nr,** nerve ring; **odr,** odontophore retractors; **oeo,** oesophageal opening; **pdg,** opening of posterior duct of digestive gland; **pma,** posterior mixing area; **poe,** posterior esophagus; **pr,** proboscis; **prr,** proboscis retractors; **r,** radula; **rd,** rhynchodeum; **sd,** salivary duct; **sg,** salivary gland; **tfl,** typhlosole; **vl,** valve of Leiblein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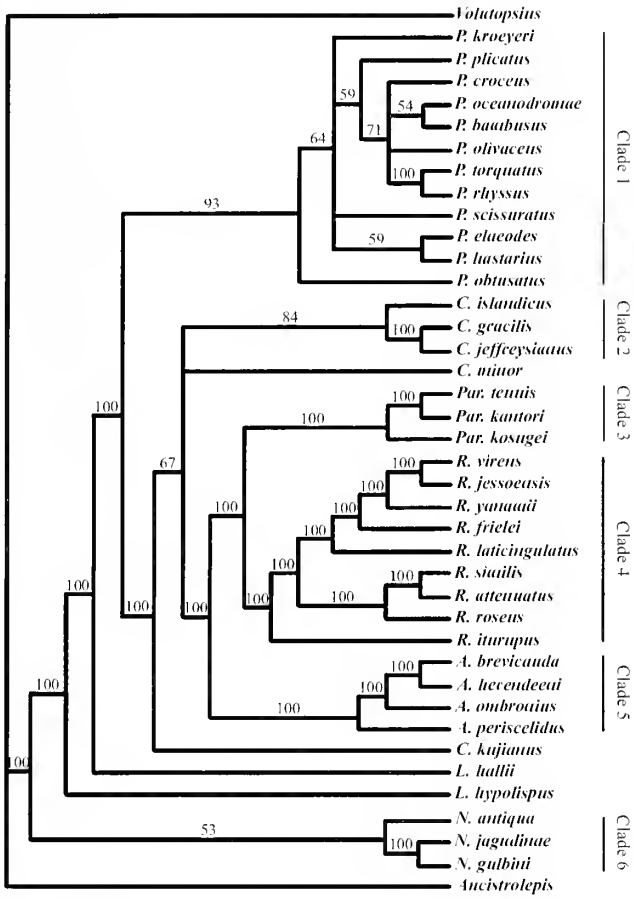


Figure 25. Fifty-percent majority-rule consensus tree obtained from 2624 trees, each 147 steps in length.

periscelidus; however, the anatomy of *Pararetifusus* differs considerably.

Clade 4, which is conchologically most heterogenous, contains 9 species previously classified within the genera *Retifusus*, *Molnia*, *Retimolnia*, and *Plicifusus*. The oldest valid name for this group is *Retifusus*.

Retifusus Dall, 1916

Plicifusus (Retifusus) Dall, 1916: 8.

Type Species: *Tritonium (Fusus) jessoensis* Schrenck, 1863 (by original designation).

Diagnosis: The genus is characterized by a small (on average < 2.5 cm) shell, which has an axial and spiral sculpture similar to that of *Plicifusus* (Figures 32, 34); however, the radula has a different morphology (Figure 37, 38). The lateral teeth usually have three or four long cusps of nearly equal length. The central teeth may be of two types. *R. jessoensis*, *R. virens*, *R. yamamii*, and *R. frielei* have five or six sharp cusps increasing in length from the periphery to the center (Figure 37). The central teeth of *R. roseus*, *R. laticingulatus*, *R. similis*, *R. iturupus*, and *R. attenuatus* have only three sharp cusps, and the central cusp is usually longer than the lateral cusps (Figure 38). The salivary ducts are very thick and straight. The stomach is large compared to the proboscis, narrow, and has a small posterior mixing area.

Remarks: McLean (1995) established the genus *Retimolnia* (type species by original designation, *Molnia frielei* (Dall, 1891) to incorporate several species previously assigned to the genus *Molnia* Friele, 1878. Our analysis demonstrates that *M. frielei* belongs to the same clade and is morphologically rather similar to *R. jessoensis*, the type species of *Retifusus*. Thus, *Retimolnia* is a junior subjective synonym of *Retifusus*. *Retifusus* is often considered to be a subgenus of *Plicifusus* (e.g., Higo et al., 1999) but our analysis demonstrates that it is not closely related to the latter.

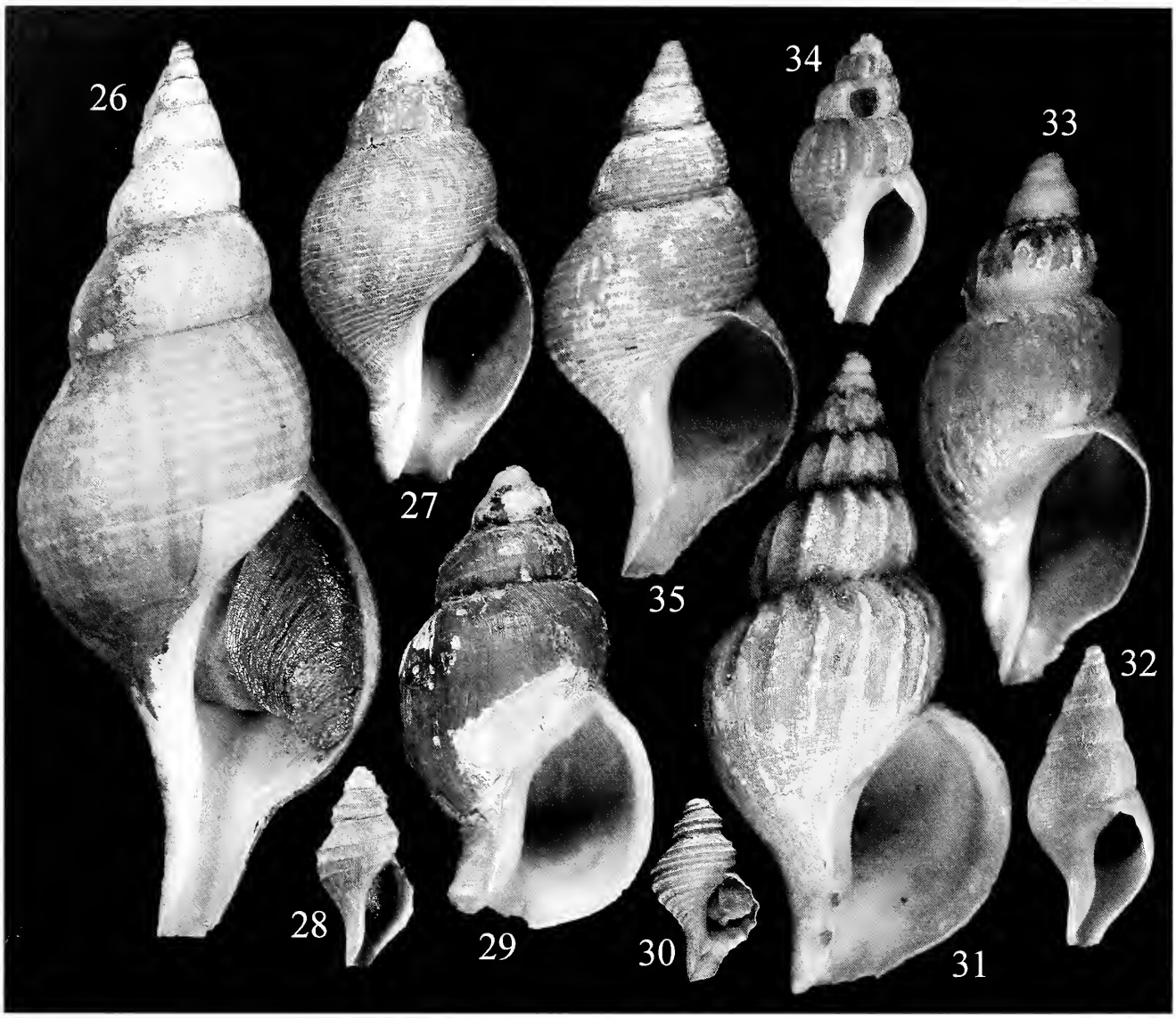
Genus Composition: We include the following species in *Retifusus*, although some others may belong to this group as well:

Retifusus jessoensis (Schrenck, 1863) [= *Fusus (Sipho?) mandhuricus* E. A. Smith, 1875; *Chrysodomus brunneus* Dall, 1877; *Molnia okhotskana* Tiba, 1981 – synonymy based on examination of the type specimens and anatomical studies .]
Retifusus frielei (Dall, 1891)
Retifusus virens (Dall, 1877)
Retifusus yamamii (Yokoyama, 1926)
Retifusus latieingulatus Golikov et Gulbin, 1977
Retifusus roseus (Dall, 1877) [= *Retifusus semiplicatus* Golikov *in* Golikov and Scarlato, 1985; *Plieifusus parvus* Tiba, 1980; *Plicifusus saginatus* Tiba, 1980 – synonymy based on examination of the type specimens and anatomical studies].
Retifusus similis (Golikov et Gulbin, 1977)
Retifusus attenuatus (Golikov et Gulbin, 1977)
Retifusus iturupus (Golikov et Sirenko, 1998)

Retifusus differs from *Plieifusus* in radular morphology; from *Molnia* in the form of its operculum, the presence of axial sculpture and in radular morphology; from *Colus*, *Aulacofusus*, and *Latisipho* in axial sculpture and radular morphology.

Clade 5 includes representatives of *Aulacofusus* that are rather uniform conchologically and morphologically.

Figures 18–24. Anatomy. **18.** Transverse section of the proboscis wall of *Aulacofusus hecendecui*. **19, 20.** Transverse section of the proboscis wall of *Aulacofusus brevicauda*. **21.** Salivary ducts of *A. brevicauda*. **22.** Transverse section of the proboscis wall of *Retifusus jessoensis*. **23.** Salivary duct of *R. jessoensis*. **24.** Transverse section of the proboscis wall of *Plicifusus hastarius*. Abbreviations: **aoe,** anterior esophagus; **cm,** circular muscles; **cnt,** connective tissue; **crt,** odontophoral cartilage; **ep,** epithelium; **lm,** longitudinal muscles; **n,** nerves; **r,** radula; **sd,** salivary duct.



Figures 26–35. Shells. **26.** *Colus islandicus*. **27.** *Latisipho hallii*. **28.** *Pararetifusus tenuis*. **29.** *Latisipho hypolispus*. **30.** *Pararetifusus kantori*. **31.** *Plicifusus kroeyeri*. **32.** *Retifusus attenuatus*. **33.** *Plicifusus rhyssus*. **34.** *Retifusus jessoensis*. **35.** *Aulacofusus brevicauda*.

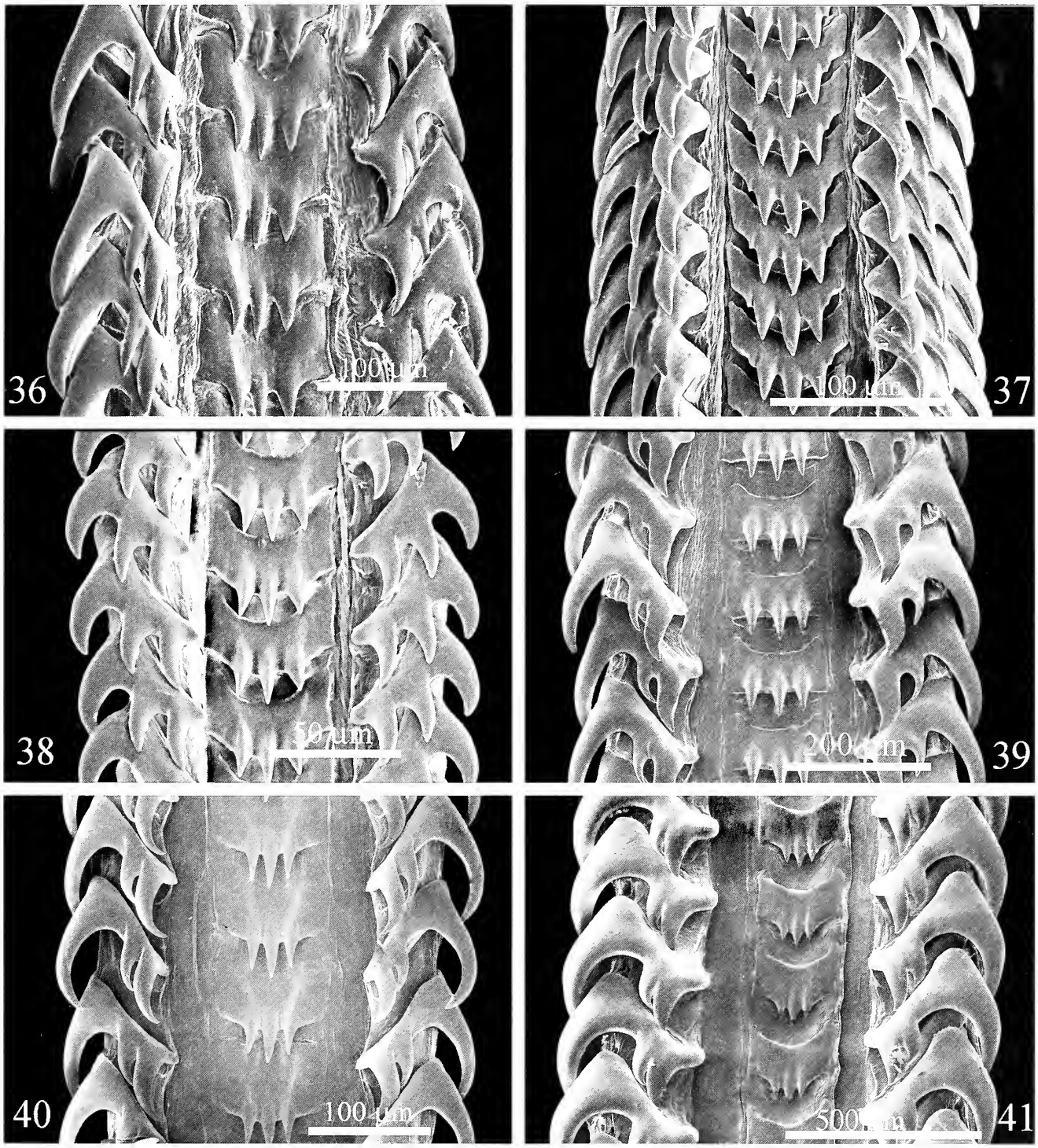
Aulacofusus Dall, 1918

Aulacofusus Dall, 1918: 217.

Type Species: *Fusus spitzbergensis* Reeve, 1855 (by original designation).

Diagnosis: The group is characterized by an elongated, medium-sized fusiform shell seulptured with wide spiral cords (from 6 to 16 cords on the penultimate whorl) (Figure 35). The axial seulpture is represented only by incremental growth lines. The radula strueture is in general the same as in *Plicifusus* (Figure 39). The salivary ducts are thick-walled, with an additional external layer of longitudinal muscles (Figure 21, **lm**). The stomach is large, as compared to the proboseis, and narrow, with a very long posterior mixing area (Figure 16, **pma**).

Remarks: The taxon was proposed as "group of species, typified by *Fusus spitzbergensis* Reeve that has a speeial aspeet due to the short canal and the prominence of the spiral ribs..." Thus, the rank of the taxon was not speeified, but it is obvious, from the context of the deseription, that Dall (1918) eonsidered it even lower than that of a section of the genus *Colus*. Later, Dall (1921) treated it as subgenus of *Colus*, a view that has been followed by most recent authors (e.g., Higo et al., 1999), but not by some Russian researchers (e.g., Golikov and Gulbin, 1977; Kantor and Sysoev, 2005, 2006).



Figures 36–41. Radulae. **36.** *Plicifusus kroeyeri*. **37.** *Retifusus jessoensis*. **38.** *Pararetifusus kantori*. **39.** *Aulacofusus brevicauda*. **40.** *Latisipho hypolispus*. **41.** *Colus islandicus*.

Species of *Aulacofusus* have a considerable conchological similarity to species attributed to the genus *Colus*, particularly in the shape and sculpture of the shell (Figures 26, 35). Some anatomical characters, such as the extremely long, coiled proboscis typical of *Aulacofusus* (Figure 14), are also present in some species of *Colus*. Nevertheless, the presence of several autapomorphies of *Aulacofusus*, including stomach structure that is

Table 1. Character coding (see Appendix 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Volutopsius norvegicus	0	0	1	0	1	0	2	0	0	?	1	1	2	1	?	1	?	0	0	1	0	0	2	2	0	1	0	1	1	2	2	1	0	0
Plicifusus krocyeri	0	1	0	0	0	0	0	0	0	0	0	0	1	0	?	?	?	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0
Plicifusus plicatus	0	1	0	0	0	0	0	0	0	?	0	0	0	0	?	0	?	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Plicifusus croccus	0	1	0	0	0	0	0	0	0	?	0	0	0	0	?	0	?	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Plicifusus scissuratus	0	1	0	0	0	0	0	0	0	?	0	0	0	0	?	0	?	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Plicifusus clacodes	0	1	0	1	0	0	0	0	0	?	0	0	0	0	?	0	?	0	0	1	0	0	2	1	2	0	0	0	0	0	1	1	0	0
Plicifusus occanodromae	0	1	1	0	0	?	?	0	?	0	0	0	0	0	?	0	?	0	0	1	2	1	1	0	1	0	0	0	0	0	1	0	0	0
Plicifusus hastarius	0	1	0	0	0	?	?	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	1	0	0
Plicifusus bambusus	0	1	1	0	0	?	?	0	?	0	0	0	0	0	?	0	?	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Plicifusus olivaccus	0	1	0	0	0	0	0	0	0	?	0	0	1	0	?	0	?	0	0	0	0	1	1	1	1	1	0	1	1	0	1	0	0	0
Plicifusus obtusatus	0	0	1	0	0	0	0	0	0	?	0	0	0	0	?	0	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0
Plicifusus torquatus	0	0	0	0	0	?	?	0	0	?	0	0	0	0	?	0	?	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0
Plicifusus rhyssus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0
Colus islandicus	0	0	0	0	0	1	2	0	1	?	0	0	0	0	?	0	?	0	1	1	?	?	2	2	0	0	0	0	0	0	1	0	0	0
Colus kujianus	0	?	?	?	?	0	0	0	0	?	0	0	1	0	?	0	?	0	1	1	0	1	2	2	0	0	0	0	0	0	1	0	0	0
Colus minor	0	0	?	?	?	0	0	0	0	?	0	0	0	0	?	0	?	0	1	0	?	?	2	2	0	0	0	0	0	0	1	0	0	0
Colus gracilis	0	0	0	0	1	1	0	0	1	?	1	0	2	0	?	1	?	0	1	0	2	0	2	2	0	0	0	0	0	0	1	0	0	0
Colus jeffreysianus	0	0	0	0	1	?	?	0	?	?	1	0	2	0	?	1	?	0	1	0	2	0	2	2	0	0	0	0	0	0	1	0	0	0
Pararctifusus tenuis	1	0	1	1	1	?	?	0	?	1	0	0	3	0	?	0	?	0	1	0	0	1	2	2	2	0	1	1	1	0	0	0	1	0
Pararctifusus kantori	1	0	1	1	0	2	2	0	0	0	0	0	3	0	?	0	?	0	1	0	?	?	2	2	2	0	2	1	1	0	0	1	1	0
Pararctifusus kosugei	0	0	1	1	0	?	?	0	?	1	0	0	0	1	?	0	?	1	1	0	0	0	2	2	2	0	1	1	1	0	0	0	1	0
Retifusus virens	2	0	0	1	0	?	?	0	?	1	0	0	0	0	?	0	?	0	1	1	0	1	0	1	1	1	0	1	1	1	2	0	1	1
Retifusus laticingulatus	2	0	1	1	1	?	?	0	?	1	0	0	0	0	?	0	?	0	0	1	?	?	0	1	1	0	0	1	1	0	0	0	1	1
Retifusus similis	2	0	1	1	0	?	?	0	?	?	0	0	0	0	?	1	?	0	1	1	0	1	0	2	1	0	0	0	0	0	0	0	1	0
Retifusus attenuatus	2	0	1	1	0	1	?	0	?	?	0	0	0	0	?	1	?	0	1	1	0	0	2	2	1	0	0	1	1	0	0	0	1	0
Retifusus yanamii	0	0	1	1	0	1	0	0	0	0	0	0	0	0	?	0	?	0	1	0	0	0	0	1	1	1	0	1	1	1	2	0	1	1
Retifusus jessocnsis	2	1	1	1	0	1	0	0	0	?	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	1	1	1	2	0	1	1
Retifusus friclci	1	1	1	1	0	1	0	0	0	?	0	0	1	0	?	0	?	0	1	0	0	0	0	1	1	0	0	1	0	1	2	0	0	1
Retifusus iturupus	0	0	1	1	0	1	?	0	0	?	0	0	0	0	?	0	?	0	0	0	?	?	0	1	1	0	0	1	1	0	0	0	1	0
Retifusus roseus	2	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	0	1	1	0	0	0	1	0
Aulacofusus brevicauda	0	0	0	1	0	?	?	0	?	1	1	1	2	1	0	1	1	0	1	1	1	0	2	2	1	0	0	0	0	0	1	0	0	0
Aulacofusus herendeeni	0	0	0	1	0	2	2	0	0	?	1	1	2	1	?	0	1	0	1	1	1	0	2	2	1	0	0	0	0	0	1	0	0	0
Aulacofusus ombronius	0	1	0	1	0	?	?	0	?	0	1	1	2	1	?	0	1	0	1	1	1	0	2	2	2	0	0	0	0	0	1	0	0	0
Aulacofusus periscelidus	0	0	0	1	0	?	?	0	0	0	1	1	2	1	?	1	1	0	1	0	1	0	2	2	2	0	3	0	0	0	1	0	0	0
Latisipho hypolispus	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	2	2	2	0	1	1	1	0	1	1	0	0
Latisipho hallii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	2	0	0	0	1	1	0	1	1	0	0
Ancistrolepis okhotensis	0	0	1	0	1	0	2	0	0	?	1	1	2	1	0	1	?	0	0	1	0	0	2	2	0	1	1	1	1	2	2	1	0	0
Neptunea antiqua	0	1	0	0	1	2	2	1	0	?	0	0	0	0	?	1	?	0	0	1	0	1	2	2	0	1	0	1	1	0	0	1	1	0
Neptunea jagudinae	0	0	1	1	1	2	2	1	0	?	0	0	0	0	?	1	?	0	0	2	?	?	2	2	0	1	0	1	1	0	0	0	0	0
Neptunea gulbini	0	0	0	0	0	0	0	1	0	?	0	0	0	0	?	1	?	0	0	2	0	1	2	2	0	1	0	1	1	0	0	0	0	0

unique in the entire subfamily Colinae, and the histological strueture of the wall of the salivary duets, lead us to treat it as a separate genus.

Genus Composition: Many species has been attributed to this group at various times. We include the following examined speeies in the subgenus:

Aulacofusus brevicauda (Deshayes, 1832) (=*Tritonium schantaricum* Middendorff, 1849; *Neptunea (Sipho) terebralis* Gould, 1860)

Aulacofusus herendeeni (Dall, 1899) (=*Colus (Aulacofusus) nobilis* Dall, 1919)

Aulacofusus ombronius (Dall, 1919)

Aulacofusus periscelidus (Dall, 1891)

Clade 6 is the most basal elade in our study, and is supported in only 53% of the trees. It includes three speeies of the genus *Neptunea* Röding, 1798: *Neptunea antiqua* (Linnaeus, 1758) (type speeies of the genus by subsequent designation of Sandberger, 1861), *N. jagudinae* Goryachev and Kantor, 1983, and *N. gulbini* Goryaehev and Kantor, 1983. The genus was ineluded in the analysis based on published data (Goryachev and Kantor, 1983) and its detailed deseription is beyond the scope of the current paper. Nevertheless, our analyses suggest that the genus in its conventional sense may be paraphyletie.

Both known species previously referred to *Latisipho* (Kosyan, 2006b) (Figures 27, 29), do not emerge as a

monophyletic group in our study, and their taxonomic position should be reconsidered.

Our study indicates that the anatomical characteristics are important and suitable for differentiating among the genera of Colinae and Buccinidae. Despite the absence, in many cases, of autapomorphies, many closely related genera may be diagnosed by combinations of characters through the use of phylogenetic techniques.

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APPENDIX 1. List of characters and character states.

Cephalopodium

1. Operculum: 0 — with terminal nucleus (Figure 1), 1 — with spiral nucleus (Figure 4), 2 — with terminal nucleus displaced to the left.

Mantle

2. Mantle: 0 — square, 1 — length exceeds width.
3. Osphradium: 0 — symmetrical, 1 — asymmetrical.
4. Osphradium: 0 — short (< ½ of mantle length), 1 — long (> ½ of mantle length).
5. Ctenidium: 0 — lamellae of ctenidium wider than lamellae of osphradium, 1 — lamellae of ctenidium of the same width as lamellae of osphradium.

Reproductive System

6. Penis: 0 — with large seminal papilla (Figures 6, 8, 9), 1 — with small papilla (Figure 10), 2 — without papilla (Figure 5).
7. Seminal papilla: 0 — cone-shaped, encircled by fold of skin (Figures 6, 8, 9), 1 — claw-like, 2 — absent.
8. Male genital opening: 0 — not surrounded by tiny papillae, 1 — surrounded by multiple tiny papillae.
9. Vas deferens: 0 — thin, convoluted, not protruding into body haemocoel, 1 — thick, located in body haemocoel.

10. Capsule gland: 0 — with ventrally folded vagina, 1 — with terminal vagina.

Digestive System

11. Proboscis: 0 — straight (Figures 12–13, pr), 1 — folded within rhynchocoel (Figure 14, pr).
12. Rhynchodeum: 0 — thick-walled, everting, 1 — thin-walled, non-everting.
13. Relative length of buccal mass: 0 — equal in length to contracted proboscis, 1 — half the length of the contracted proboscis, 2 — less than half the length of the contracted proboscis, 3 — longer than the contracted proboscis.
14. Proboscis retractors: 0 — running along rhynchodeum and attached to roof and lateral walls of body haemocoel (Figure 12–13, prr), 1 — short, situated at the base of the proboscis and attached to the bottom of body haemocoel (Figure 14, prr).
15. Sequence of layers in the proboscis wall [outer to inner edges]: 0 — epithelium, circular muscles, longitudinal muscles, circular muscles, longitudinal muscles (Figure 19), 1 — epithelium, longitudinal muscles, circular muscles, longitudinal muscles, circular muscles (if present) (Figure 24).
16. Salivary glands: 0 — small and rounded (< 1/3 of proboscis length) (Figure 12), 1 — long, bean-shaped (> 2/3 of proboscis length) (Figures 13, 14).
17. Salivary ducts: 0 — without additional longitudinal muscle layer in the wall (Figures 22, 23), 1 — with external layer of longitudinal muscles in the wall (Figures 20, 21).
18. Salivary ducts: 0 — without salivary sacs (Figures 13, 14), 1 — with salivary sacs (Figure 15).
19. Salivary ducts: 0 — thin, convoluted (Figure 13), 1 — thick, straight (Figures 14, 15).
20. Gland of Leiblein: 0 — well developed, 1 — thin, poorly developed, 2 — absent.
21. Stomach: 0 — with small posterior mixing area (Figure 17), 1 — with very long posterior mixing area (Figure 16), 2 — without posterior mixing area.
22. Stomach: 0 — large (>1/3 whorl), 1 — small (<1/3 whorl).

Shell

23. Axial ribs: 0 — < 14 axial ribs on last whorl, 1 — > 14 ribs on last whorl, 2 — axial ribs absent.
24. Axial ribs: 0 — s-shaped, 1 — straight, 2 — absent.
25. Spiral sculpture: 0 — numerous cords present (> 20 on penultimate whorl), 1 — few cords present (< 20 on penultimate whorl), 2 — cords absent.
26. Microscopic spiral threads: 0 — present, 1 — absent.
27. Spiral cords: 0 — absent, 1 — present, low, acute distally, 2 — present, rounded distally, 3 — present, flattened.
28. Ratio, body whorl height / shell height: 0 — <0.7; 1 — >0.71.
29. Ratio, aperture length / shell length: 0 — <0.5; 1 — >0.51.

Radula

30. Central tooth: 0 — with 3 cusps (Figures 36, 38–41), 1 — with multiple cusps, posterior tooth edge rounded (Figure 37), 2 — with multiple cusps, posterior tooth edge nearly straight.
31. Central tooth: 0 — with 3 cusps, all of equal size, 1 — with 3 cusps, medial cusp differing in size from the marginal cusps, 2 — with more or fewer than 3 cusps.
32. Lateral teeth: 0 — with 3 cusps, 1 — with more or fewer than 3 cusps.
33. Lateral teeth: 0 — medial cusps smallest, 1 — all cusps equal in length.
34. Cusps of the central tooth: 0 — do not overlap tooth of following row; 1 — overlap tooth of following row.

The anatomy and relationships of *Troschelia* (Neogastropoda: Buccinidae): New evidence for a closer fasciolariid-buccinid relationship?

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ABSTRACT

Analyses of new anatomical and molecular data confirm the taxonomic position of *Troschelia berniciensis* (King, 1846) within the Buccinidae and provide the framework for a review of the relationships of the families Fasciolariidae and Buccinidae. The morphology of *Troschelia* Mörch, 1876, is similar to that of other Northern Atlantic and Pacific buccinid genera. Anatomical examination of a number of fasciolariid species revealed only a single character, the structure of the proboscis retractor muscles, to be diagnostic of the Fasciolariidae, while other characters are shared with the Buccinidae. A molecular phylogeny also confirms a close relationship between the two groups.

Additional keywords: Taxonomy, phylogeny, anatomy, Buccinidae, Fasciolariidae

INTRODUCTION

Phylogenetic relationships among the more than 200 genera and subgenera included in the gastropod family Buccinidae remain quite ambiguous. The northeastern Atlantic monotypic genus *Troschelia* Mörch, 1876, has been classified by a number of authors (e.g., G.O. Sars (1878), J. Thiele (1929)) in the family Fasciolariidae, due to the peculiar radula of *Troschelia berniciensis* (King, 1846). However, Bouchet and Warén (1985) placed *Troschelia* within Buccinidae, based on the morphology of its lateral teeth, which have multiple, uniform cusps similar to those of some other Buccinidae (*Thalassoplanes* Dall, 1908). To elucidate the phylogenetic relationships of *Troschelia* with the families Buccinidae and Fasciolariidae, morphological and anatomical features were studied, and partial sequences from the mitochondrial 16S rRNA gene were analyzed for *Troschelia berniciensis* as well as for a number of buccinid and fasciolariid taxa.

MATERIALS AND METHODS

Samples for the present study were collected during field work and expeditions to the West Pacific (Panglao 2004, Philippines, and Santo 2006, Vanuatu, organized by the Muséum national d'Histoire naturelle, Paris), to Panama (Neogastropod Workshop 2006, at the Smithsonian Tropical Research Institute, Panama), the Mediterranean Sea, and at other localities, and supplemented by specimens provided by Museums (BMNH – Natural History Museum, London, UK, MNHN – Muséum National d'Histoire naturelle, Paris, France, BAU – Museum of Biologia Animale e dell'Uomo Department, University "La Sapienza", Rome, Italy) and colleagues. The taxa listed in Table 1 were used for anatomical studies. Animals were dissected and radulae examined using an SEM. Sequence data was newly generated for several species, and supplemented with additional taxa that were obtained from GeneBank (see Table 2).

DNA Extraction, PCR, and Sequencing: Total DNA was extracted following a standard Phenol/Chloroform/Ethanol protocol (Hillis et al., 1990) with slight modification as previously described by Oliverio and Mariottini (2001) for mollusks. QIAGEN QiAmp Extraction Kit was used according to manufacturer's instructions for extraction of DNA from difficult samples.

A region of the gene encoding 16SrDNA encompassing the domains IV and V (Gutell and Fox, 1988) was amplified using primers 16SA (5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi et al., 1991) and 16SH (5'-CCGGTCTGAACTCAGATCAC-3') (Espiritu et al., 2001). Amplification conditions were as follows (30–35 cycles): 94° for 30 sec, 45–50°C for 30 sec, 72°C for 60 sec. When a single band was obtained the PCR product was purified using the Exo-Sap enzymatic method. Purified products were then double strand sequenced with BigDye v. 2.0 (Applied Biosystems, Foster City, CA, USA)

Table 1. Species used in the anatomical study.

Family	Subfamily	Species	Locality	Voucher number
Buccinidae		*Troschelia berniciensis* (King, 1846)	Station number: 44/2, Sea Area: S38, 51°19.2′ N, 08°32.2′ W, Depth: 95m, Date: 28/05/1975 RRS CHALLENGER Cruise 8/75	BMNH 20031298 BAU00687
Fasciolariidae	Peristerninae	*Turrilatirus turritus* (Gmelin, 1791)	Sudan, Red Sea, FEL-93 Expedition, Sha'ab Rumi, Stn. 4, N 6, 0.5–1 m, reef-flat brushing, pocilloporids and coral rubble, M/S FELICIDAD	BAU00691
Fasciolariidae	Peristerninae	*Pustulatirus mediamericanus* (Hertlein and Strong, 1951a),	Panama, Pacific Ocean, Venado beach, 8.89° N, 79.59° W, intertidal, Feb. 2006	BAU00688
Fasciolariidae	Peristerninae	*Latirus polygonus* (Gmelin, 1791)	Sudan, Red Sea, REDSED–5 Cruise, Sanganeb Reef, Stn. RS5–5, N 1, Southern Lagoon, sand and patchy reefs, 2 m, M/S FELICIDAD – BIORES group, Leg. M. Oliverio, M. Taviani, 3 Sep.1993	BAU00690
Fasciolariidae	Peristerninae	*Peristernia nassatula* (Lamarck, 1822)	Sudan, Red Sea, REDSED-5 Cruise, Sanganeb Reef, Stn. RS5-3, N 1, lagoon plus reef flat, brushing coral rabbish and direct search in situ, 2 Sep. 1993 day – 9 Sep. 1993 night	BAU00692
Fasciolariidae	Peristerninae	*Peristernia ustulata* (Reeve, 1847)	Philippines, Panilacan, 9.48° N, 123.9°3 E, 10–41 m	BAU00693
Fasciolariidae	Peristerninae	*Opeatostoma pseudodon* (Burrow, 1815)	Panama, Pedro Gonzales Island, Archipelagus Las Perlas, 8.4° N 79.1° W, intertidal, 02.2006	BAU00689
Fasciolariidae	Fusininae	*Fusinus tenerifensis* Hadorn and Rolán, 1999	Canary Islands, Puerta de la Cruz	BAU00694
Fasciolariidae	Fasciolariinae	*Fasciolaria lignaria* (Linnaeus, 1758)	S. Marinella (Italy) 42°02′ N 11°54′ E, intertidal	BAU00227

using the PCR primers and sequences visualized on automatic sequencer. Sequencing was performed by Macrogen Inc. (Seoul, South Korea). Chromatograms were analysed by Staden Package (Version 1.6.0, Staden et al., 1998, 2005). All sequences have been deposited at EMBL (see Table 2 for accession numbers).

Sequences obtained were aligned using ClustalX (Thompson et al., 1994; 1997) with the default settings. The alignments obtained were manually edited. The χ^2 test implemented in PAUP* v. 4b10 (Swofford, 2002) was used to test for base composition homogeneity of the sequence data aligned.

A Bayesian analysis of the aligned sequences was performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003), which sampled trees from posterior densities using the Markov Chain Monte Carlo method (Larget and Simon, 1999; Yang and Rannala, 1997). The substitution model to be used in the Bayesian analysis was chosen after evaluation by the software MrModeltest 2.2 (Nylander, 2004), while base frequencies, relative rates of the six substitution types and model parameters were estimated by MrBayes during phylogenetic reconstruction. A four-chain metropolis-coupled Monte Carlo analysis was run twice in parallel for 10^6 generations, and trees were sampled every 1000 generations, starting after a burn-in of 250000 generations. Stationarity was considered to be reached when the average standard deviation of split frequencies shown in MrBayes was less than 0.01 (Ronquist and Huelsenbeck, 2003). Bayesian posterior probabilities (BPP) of a branch were estimated as the percentage of trees (after burn-in) which showed that specific node.

RESULTS

Anatomy of *Troschelia berniciensis:* EXTERNAL MORPHOLOGY: Animal (Figures 1–3) is uniform cream in color. Foot (Figure 1, **ft**) partly contracted, with deep propodial groove (**prpg**) separating narrow propodium. Operculum oval, with terminal nucleus (Figure 1, **op**). Head moderately large, broad (Figure 1, **hd**) with pair of long, thick tentacles, each with large black eye on

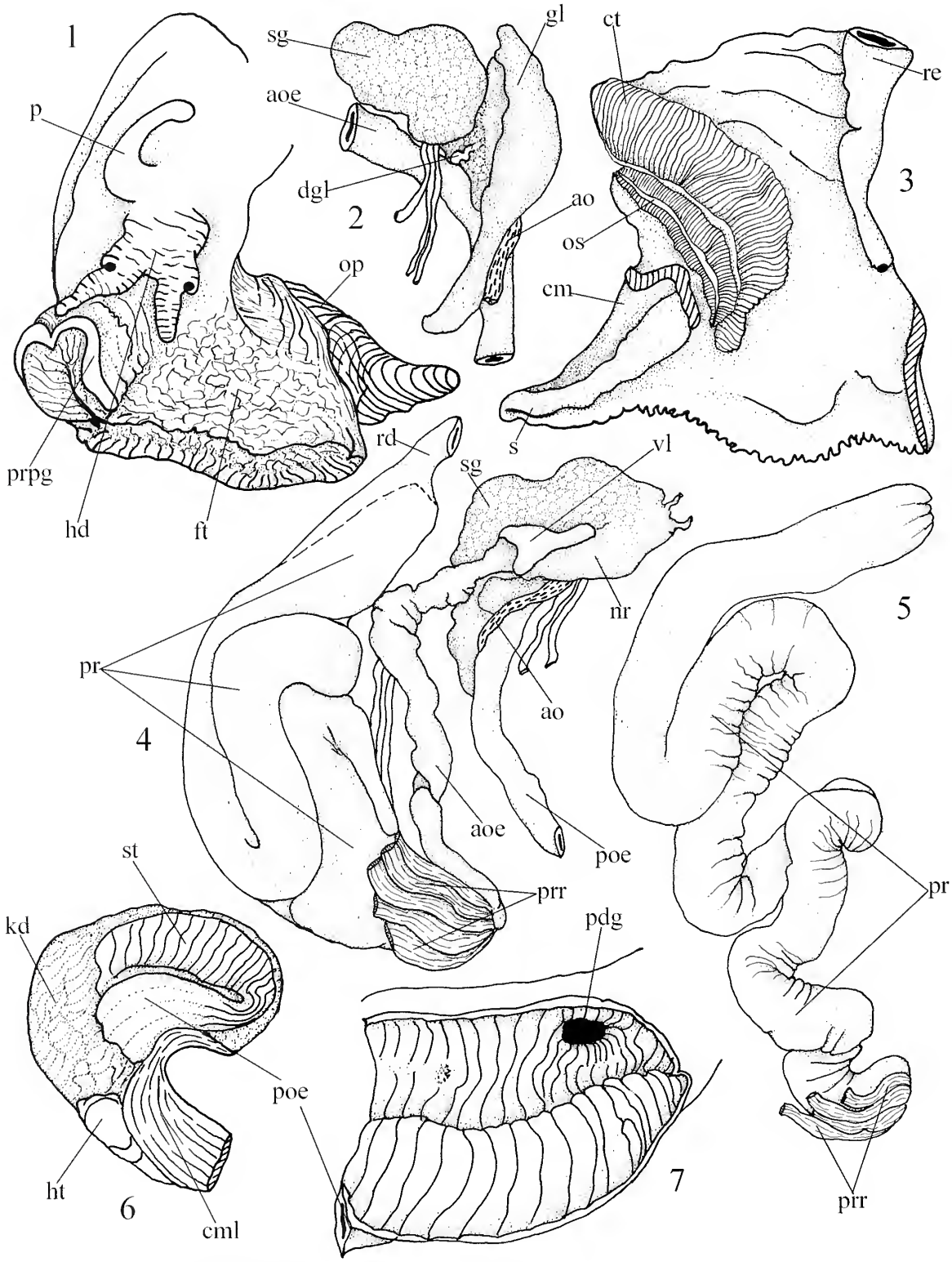
Table 2. Species included in the phylogeny based on partial 16S DNA sequences.

Family	Species	Locality	Voucher number	EMBL	Reference
Cancellariidae	*Cancellaria cancellata* Linné, 1767	Off Malaga (Spain), 40–50 m	BAU00224	FM999105	Oliverio and Modica, in press
Fasciolariidae	*Turrilatirus turritus* (Gmelin, 1791)	Balicasag (Philippines) 9.51° N, 123.68° E, 80–150m	BAU00695	FN394061	this study
Fasciolariidae	*Fasciolaria lignaria* (Linnaeus, 1758)	S. Marinella (Italy) 42.03° N, 11.9° E, intertidal	BAU00227	FN394059	this study
Fasciolariidae	*Fusinus akitai* Kuroda and Habe in Habe, 1961	Off Atsumi, Aichi, central Japan	—	AB044253	Hayashi, 2005
Fasciolariidae	*Granulifusus niponicus* (E.A. Smith, 1879)	Off Kushimoto, Wakayama, central Japan	—	AB044254	Hayashi, 2005
Buccinidae	*Troschelia berniciensis* (King, 1846)	South of Ireland, 51°19.2′ N, 08°32.2′ W	BMNH 20031298 BAU00687	FN394057	this study
Buccinidae	*Pollia tincta* Conrad, 1846	St. Petersburg Beach, Pinellas, Florida, USA	—	AB044270	Hayashi, 2005
Buccinidae	*Pisania striata* Gmelin, 1791	Salina Is. (Italy), 38.58° N, 14.80° E, intertidal	BAU00698	FM999128	Oliverio and Modica, in press
Buccinidae	*Engina pulchra*	Venado (Panama), 8.89° N, 79.59° W intertidal	BAU00276	FN394058	this study
Buccinidae	*Cantharus multangulus* (Philippi, 1848)	Tierre Verde, Pinellas, Florida, USA	—	AB044259	Hayashi, 2005
Buccinidae	*Paracanthria plumbea* (Philippi, 1841)	Ushuaia (Argentina), 54.78° S, 68.23° W, intertidal	BAU00697 MNHN IM-2009-4613	FM999126	Oliverio and Modica, in press
Buccinidae	*Neobuccinum eatoni* (Smith, 1875)	Terra Nova Bay (Antarctic), 74.69° S, 164.12° E,	BAU00785 MNHN IM-2009-4614	FM999127	Oliverio and Modica, in press
Buccinidae	*Chlanidota densesculpta* Martens, 1885	South Sandwich Islands 56.24° S, 27.44° W	BAU00230	FN394060	this study
Buccinidae	*Buccinulum linea* (Martyn, 1784)	Leigh Harbour, New Zealand	—	AB044256	Hayashi, 2005
Buccinidae	*Japanitria ferrea* (Reeve, 1847)	Suga Island, Ise Bay, Mie, central Japan	—	AB044262	Hayashi, 2005
Buccinidae	*Siphonalia cassidariaeformis* (Reeve, 1843)	off Shizuoka, central Japan	—	AB044271	Hayashi, 2005
Buccinidae	*Kelletia kelletii* (Forbes, 1850)	Santa Barbara Island, Los Angeles, USA	—	AB121037	Hayashi, 2005
Buccinidae	*Penion sulcatus* (Lamarck, 1816)	Unknown, New Zealand	—	AB044267	Hayashi, 2005
Buccinidae	*Phos laeve* Kuroda and Habe in Habe, 1961	Off Kushimoto, Wakayama, central Japan	—	AB044268	Hayashi, 2005
Buccinidae	*Neptunea intersculpta* (Sowerby, 1899)	Off Hokkaido, north Japan	—	AB044265	Hayashi, 2005
Buccinidae	*Buccinum opisoplectum* Dall, 1907	unknown	—	AB044257	Hayashi, 2005

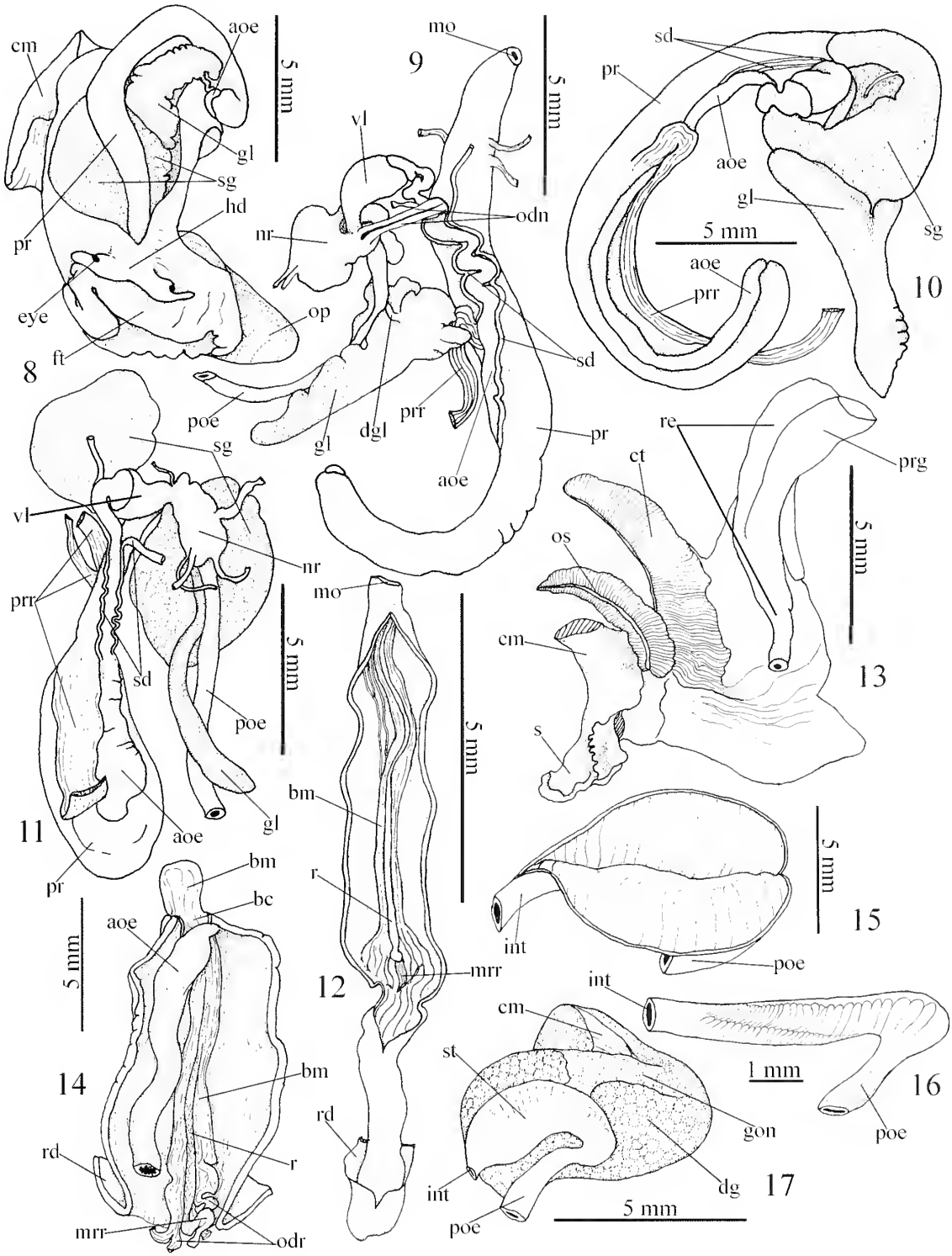
outer side of swelling at its base. Penis (Figure 1, **p**) small, apparently underdeveloped.

Mantle: Mantle margin indented (Figure 3). Siphon (Figure 3, **s**) moderately long, muscular. Osphradium (**os**) occupies ~ ½ mantle length, ~1/6 mantle width. Ctenidium (**ct**) long, crescent-shaped, occupying 4/5 mantle length. Hypobranchial gland (**hg**) not well developed.

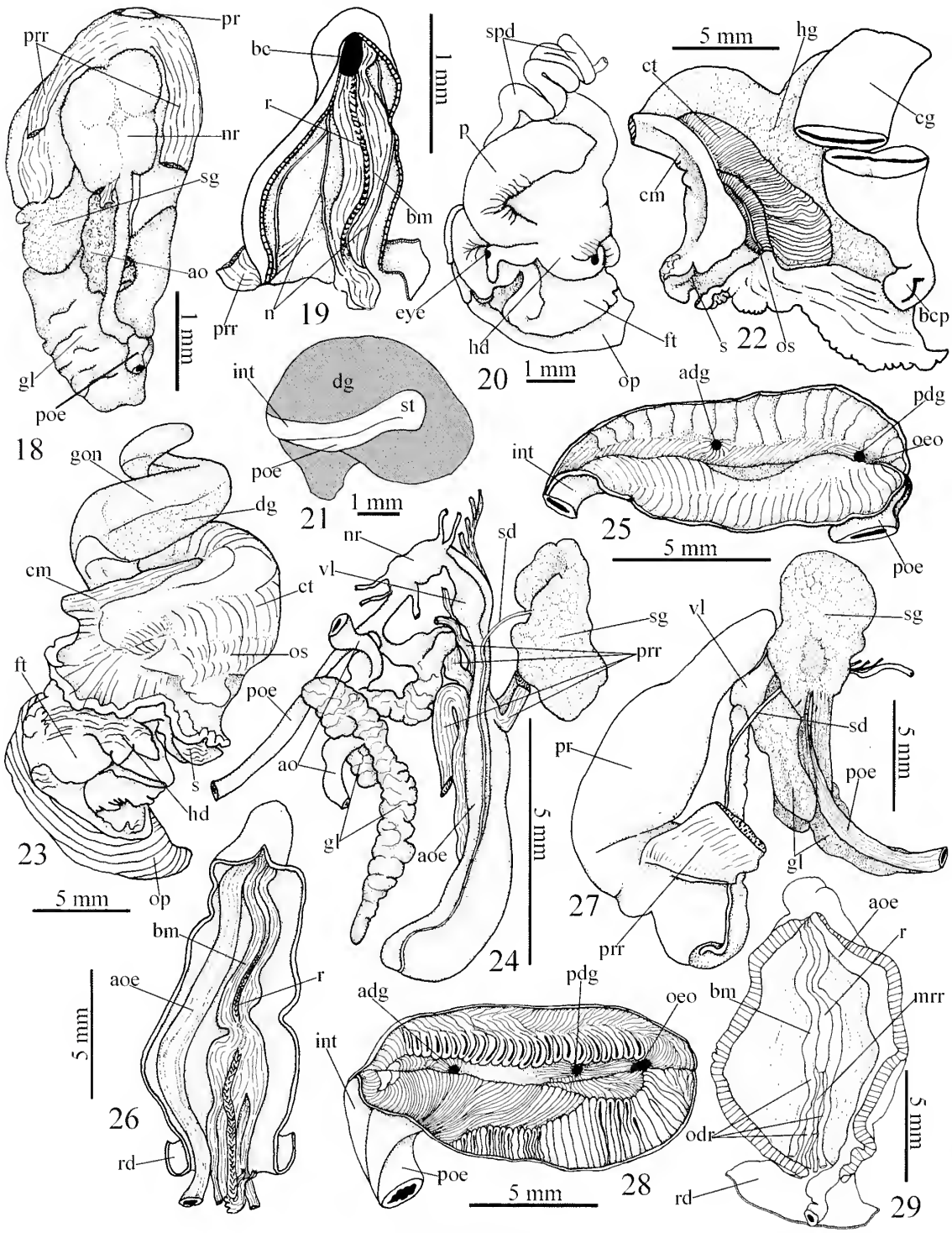
Digestive System: Proboscis extremely long, narrow (Figures 4–5, **pr**), compactly folded within rhynchodeum (Figure 4, **rd**). Buccal mass occupies ~ ¼ proboscis length. Radula equal in length to odontophore, with structure similar to that illustrated in Bouchet and Warén (1985: 184, fig. 485). Proboscis attached to bottom of body haemocoel by proboscis retractors (Figures 4–5, **prr**) emerging from its base, consisting ~ 6 multiple



Figures 1–7. Anatomy of *Troschelia berniciensis*. **1.** Cephalopodium, visceral mass removed. **2.** section of esophagus with duct of gland of Leiblein. **3.** Mantle. **4.** Foregut. **5.** Proboscis. **6.** Stomach. **7.** Internal structure of the stomach. Abbreviations: **ao,** anterior aorta, **aoe,** anterior esophagus; **cm,** columella muscle; **ct,** ctenidium; **ft,** foot; **gl,** gland of Leiblein; **hd,** head; **ht,** heart; **kd,** kidney; **nr** = nerve ring; **op,** operculum; **os,** osphradium; **p,** penis; **pdg,** posterior duct of digestive gland; **poe,** posterior esophagus; **pr,** proboscis; **prpg,** propodial groove; **prr,** proboscis retractors; **rd,** rhynchodeum; **re,** rectum; **s,** siphon; **sg,** salivary gland; **st,** stomach; **vl,** valve of Leiblein.



Figures 8–17. Anatomy of Fasciolariidae. **8–10.** *Peristernia ustulata*. **8.** Cephalopodium, visceral mass removed. **9.** Foregut, ventral view. **10.** Foregut, lateral view. **11.** Foregut of *Pustulatirus mediamericanus*, ventral view. **12.** Proboscis of *P. ustulata*. **13–15.** *Pustulatirus mediamericanus*. **13.** Mantle. **14.** Proboscis. **15.** Internal structure of the stomach. **16.** Stomach of *Latirus polygonus*. **17.** Stomach of *P. ustulata*. Abbreviations: **aoe,** anterior esophagus; **bc,** buccal cavity; **bm,** buccal mass; **cm,** columellar muscle; **ct,** ctenidium; **dg,** digestive gland; **dgl,** duct of gland of Leiblein; **eye,** eye; **ft,** foot; **gl,** gland of Leiblein; **gon,** gonad; **hd,** head; **int,** intestine; **mo,** mouth opening; **mrr,** medial retractor of radula; **nr,** nerve ring; **odn,** odontophore nerves; **odr,** odontophore retractors; **op,** operculum; **os,** osphradium; **poe,** posterior esophagus; **pr,** proboscis; **prg,** prostate gland; **prr,** proboscis retractors; **r,** radula; **rd,** rhynchodeum; **re,** rectum; **s,** siphon; **sd,** salivary duct; **sg,** salivary gland; **st,** stomach; **vl,** valve of Leiblein.



Figures 18–29. Anatomy of Faseiolariidae. **18–21.** *Fusinus tenerifensis.* **18.** Foregut. **19.** Proboseis. **20.** Cephalopodium. **21.** Stomach. **22–24.** *Opeatostoma pseudodon.* **22.** Mantle. **23.** Soft parts. **24.** Foregut. **25.** Internal structure of the stomach. **26.** Proboseis. **27–29.** *Fasciolaria lignaria.* **27.** Foregut. **28.** Internal structure of the stomach. **29.** Proboscis. Abbreviations: **adg,** anterior duct of digestive gland; **ao,** anterior aorta; **aoe,** anterior esophagus; **be,** bueeal eavity; **bcp,** bursa eopulatrix; **bm,** bueeal mass; **cg,** capsule gland; **cm,** columella musele; **et,** ctenidium; **dg,** digestive gland; **eye,** eye; **ft,** foot; **gl,** gland of Leiblein; **gon,** gonad; **hd,** head; **hg,** hypobranchial gland; **int,** intestine; **mrr,** medial retraetor of radula; **n,** nerves; **nr,** nerve ring; **odr,** odontophore retractors; **oeo,** oesophageal opening; **op,** opereulum; **os,** osphradium; **p,** penis; **pdg,** posterior duct of digestive gland; **poe,** posterior esophagus; **pr,** proboscis; **prr,** proboscis retractors; **r,** radula; **rd,** rhynchodeum; **s,** siphon; **sd,** salivary duct; **sg,** salivary gland; **spd,** spermoduct; **st,** stomach; **vl,** valve of Leiblein.

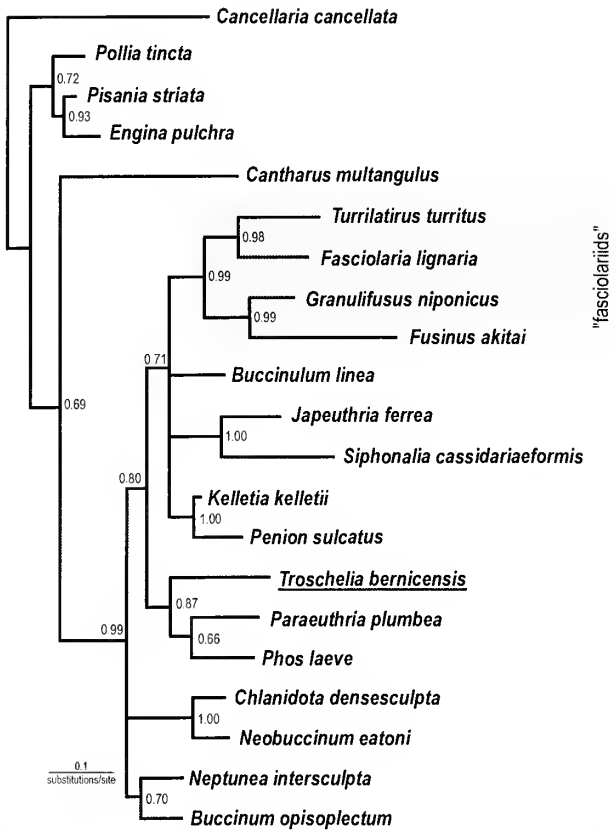


Figure 30. Bayesian topology obtained for the molecular dataset. Numbers at nodes are the Bayesian Posterior Probabilities.

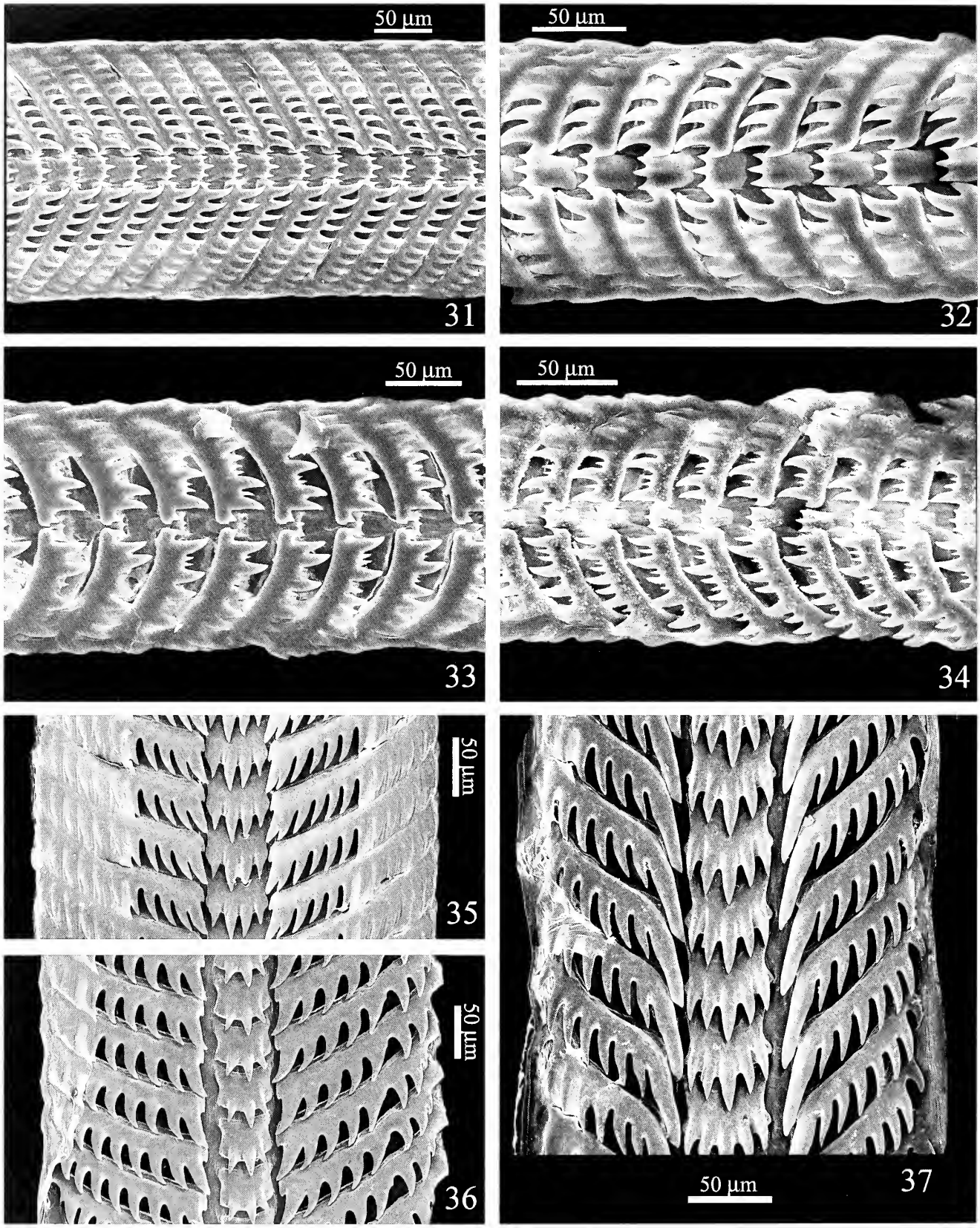
muscle tufts. Anterior esophagus wide, convoluted. Valve of Leiblein small, pyriform, situated immediately anterior to nerve ring. Salivary glands of medium size (Figures 2, 4, **sg**), tightly packed with nerve ring (Figure 4, **nr**) by connective tissue. Anterior aorta (Figure 4, **ao**) passes through nerve ring, runs parallel to posterior esophagus. Stomach narrow, occupying ~ 1/3 whorl (Figure 6, **st**). Posterior mixing area is absent, stomach walls lined with high, transverse folds of epithelium. Opening of posterior duct of digestive gland large, situated above the oesophageal opening (Figure 7, **pdg**).

Anatomy of Fasciolariid Species: Eight species of Fasciolariidae, representing seven genera from three subfamilies, were studied anatomically (Table 1). Main external morphological features included a folded, large muscular foot (Figures 8, 20, 23, **ft**), a broad head with relatively short tentacles (Figures 8, 20, 23, **hd**), and an operculum with a terminal nucleus (Figures 8, 23, **op**). The mantle (Figures 13, 22) has a moderately large, muscular siphon, a ctenidium occupying ¼–1/3 of the mantle width, and an asymmetrical osphradium that may be large (*Fusinus tenerifensis* Hadorn and Rolán, 1999, *Latirus polygonus* (Gmelin, 1791), *Pustulatirus mediamerieanus* (Hertlein and Strong, 1951a), *Faseiolaria lignaria* (Linnaeus, 1758), *Peristernia nassatula* (Lamarck, 1822), *P. ustulata* (Reeve, 1847) – Figure 13, **os**) or small (*Opeatostoma pseudodon* (Burrow, 1815) – Figure 22, **os**).

Digestive System: The proboscis is straight and never coiled within the rhynchodeum. It may be rather short in *Pustulatirus mediamerieanus* (Figure 14), *Latirus polygonus*, *Opeatostoma pseudodon* (Figure 26), *Fusinus tenerifensis* (Figure 19), and *Faseiolaria lignaria* (Figure 29), with the length of the buccal mass and radula equal to the length of proboscis (Figures 14, 19, 26, 29, **bm**). The proboscis of *Peristernia nassatula*, *P. ustulata* and *Turrilatirus turritus* (Gmelin, 1791) is very long, spanning one whorl (Figures 8–10, **pr**). The buccal mass and radula are about half of the proboscis length (Figure 12, **bm**) in these taxa. The radula of *Pustulatirus mediamerieanus* has a small, 4-cuspid rachidian tooth and broad lateral teeth with 11 cusps of equal size on the left and 12 cusps on the right of the rachidian (Figure 35). The radula of *Latirus polygonus* has a small, 3-cuspid rachidian tooth, with the median cusp slightly longer than marginal cusps; lateral teeth have 11 and 12 equal cusps on left and right longitudinal rows, respectively (Figure 31). The radula of *Turrilatirus turritus* has a similar rachidian tooth and lateral teeth with only 7 equal cusps in each row (Figure 32). *Peristernia nassatula* (Figure 34) and *P. ustulata* (Figure 33) possess very similar radulae, with very small 3-cuspid rachidian teeth and lateral teeth with multiple alternating smaller and larger cusps. The radula of *Opeatostoma pseudodon* (Figure 37) has a 5-cuspid rachidian that is unusually large for fasciolariids, and lateral teeth with 8 equal cusps in each longitudinal row. The radula of *Faseiolaria lignaria* (Figure 36) corresponds to previously published figures (see Bandel, 1984).

There is only one, long and powerful proboscis retractor muscle in *Peristernia nassatula*, *P. ustulata*, and *Turrilatirus turritus*; it emerges from the middle part of the proboscis, runs ventrally, and attaches to the columellar muscle (Figure 10, **prr**). The single proboscis retractor of *Faseiolaria lignaria* is very wide and short, starting from the posterior section of the rhynchodeum (Figure 27, **prr**). In *Pustulatirus mediamerieanus* one powerful ventral proboscis retractor is supplied by two smaller and thinner muscles, situated in the upper part of the rhynchodeum (Figure 11, **prr**). In *Opeatostoma pseudodon* there are two main lateral proboscis retractor muscles as well as several additional thin retractor muscles, situated more anteriorly (Figure 24, **prr**). *Fusinus tenerifensis* (Figure 18, **prr**) and *Latirus polygonus* possess two lateral proboscis retractor muscles.

The anterior esophagus is wide, dorso-ventrally flattened, flanked by two salivary ducts that are not embedded in its wall in all studied species (Figures 9, 11, 24, 27, **aoe, sd**) except for *Latirus polygonus*. The salivary glands (Figures 8, 10, 11, 18, 24, 27, **sg**) are large, separate in *Fusinus tenerifensis*, *Fasciolaria lignaria*, and *P. mediamericanus*, and fused beneath the nerve ring in *Turrilatirus turritus*, *Peristernia nassatula*, *P. ustulata* and *Opeatostoma pseudodon*. The



Figures 31–37. Radulae of Fasciolariidae. **31.** *Latirus polygonus*. **32.** *Turrilatirus turritus*. **33.** *Peristernia ustulata*. **34.** *Peristernia nassatula*. **35.** *Pustulatirus mediamericanus*. **36.** *Fasciolaria lignaria*. **37.** *Opeatostoma pseudodon*.

Table 3. Distinguishing anatomical features of the buccinids and fasciolariids examined in this study.

Feature Species	Proboscis	Proboscis retractors	Salivary glands	Salivary ducts	Stomach	Radula
Troschelia berniciensis	Long, coiled, buccal mass is 1/6 of pr. length	Multiple	Fused	?	Narrow, **pma** absent	5:1:5, lateral cusps of equal size
Pustulatirus mediamericanus	Short, straight, buccal mass is equal to pr. length	Single powerful and two thin additional	Fused	Free, twisting	Broad, with several medium high folds, **pma** absent	7:3:8, lateral cusps of equal size
Latirus polygonus	Short, straight, buccal mass is equal to pr. length	Paired	?	Embedded	Narrow, **pma** absent	11:3:12, lateral cusps of equal size
Turrilatirus turritus	Long, straight, buccal mass is ½ of pr. length	Single, long and narrow	Fused	Free, straight	Medium broad, **pma** absent	7:3:7, lateral cusps of equal size
Peristernia nassatula, Peristernia ustulata	Long, straight, buccal mass is ½ of pr. length	Single, long and narrow	Fused	Free, straight	Medium broad, **pma** absent	12:3:12, lateral cusps of different size
Opeatostoma pseudodon	Short, straight, buccal mass is equal to pr. length	Two powerful and several additional	Fused	Free, straight	Medium broad, with several medium high folds, **pma** absent	8:5:8, lateral cusps of equal size
Fusinus tenerifensis	Short, straight, buccal mass is equal to pr. length	Paired	Separate	?	Narrow, **pma** absent	?
Fasciolaria lignaria	Short, straight, buccal mass is 2/3 of pr. length	Single, short and broad	Separate	Free, straight	Broad, with multiple low folds, **pma** absent	12:3:12, lateral cusps of equal size

valve of Leiblein is moderately large and pyriform. The gland of Leiblein is very large in *Fusinus tenerifensis, Turrilatirus turritus,* and *Peristernia* species, and of medium size in *O. pseudodon, P. mediamericanus,* and *F. lignaria* (Figures 9–11, 18, 24, 27, **vl, gl**). The anterior aorta (**ao**) is very large and thick-walled. The stomach of all fasciolariids examined lack a posterior mixing area. They are narrow and long, with well-developed inner epithelial folds, high in *O. pseudodon* and *P. mediamericanus* (Figures 15, 25), and low in *F. lignaria* (Figure 2S). The stomachs of *O. pseudodon* and *F. lignaria* possess two openings of ducts of the digestive gland, situated a short distance from each other, A longitudinal fold (Figures 25, 28, **lfl**) is present on the inner stomach wall, as are multiple transverse folds on the outer wall. The internal stomach structure of the remaining species was not studied due to poor preservation (outer view on Figures 16, 17, 21). Anatomical features of the studied fasciolariid species are summerized in the Table 3.

Molecular Analysis: A total of 9 new, partial 16S ribosomal DNA sequences were obtained, each 487–493 bp long (including the outgroup *Cancellaria cancellata*), and analyzed together with 12 previously published buccinoidean 16S sequences (Hayashi, 2005) (See Table 2). The aligned dataset comprised 514 nucleotide positions.

A χ^2 test of base homogeneity, uncorrected for phylogeny, indicated that base composition was not significantly different across all sites (P = 0.999). The model used for Bayesian analysis was HKY+I+G, as selected by the Akaike Information Criterion in MrModeltest 2.2. In the resulting tree, *Troschelia berniciensis* occupies a basal position in a clade with *Paracuthria plumbea* and *Phos laeve* (Figure 30) ,with a bayesian posterior probability (bpp) of 0.87. This clade is the sister group to a larger, unresolved grouping, comprising several buccinid species and all the Fasciolariidae included in the analysis. The fasciolariids in this study form a well-supported monophyletic group (bpp=0.99). However, the placement of the fasciolariid clade among the buccinid taxa suggest that the Buccinidae is paraphyletic in our analysis, and that the Fasciolariidae may be a stem group within Buccinidae.

DISCUSSION

Troschelia berniciensis, though differing in radular structure, is very similar to other boreal representatives of the family Buccinidae in the morphology of its foregut, especially to Atlantic species of *Colus* (Kosyan, pers.

observ.), and to *Aneistrolepis* (Kantor, 1988). All have a long, coiled proboscis, proboscis retractors consisting of multiple tufts of muscular fibers that attach to the base of the proboscis, and a stomach without a posterior mixing area. The last feature has been considered to be typical for Fasciolariidae (Kantor, 2003), as is a radula with a small rachidian tooth and multi-cuspidate lateral teeth.

Although Ponder (1970) concluded that there are no reliable anatomical differences readily distinguishing the families included in Buccinoidea, Kantor (2003), and later Fraussen et al. (2007), reported that a combination of features, including a characteristic stomach morphology, together with multicuspid lateral radular teeth, a very small central tooth, single or paired proboscis retractor muscles, and salivary ducts passing within the esophagus walls, allows for the confident diagnosis of the family Fasciolariidae.

Our data confirm that the anatomy of the fasciolariids we studied is, in general, very similar to that of Buccinidae. Fasciolariid stomachs lack the posterior mixing area, and vary in internal structure from buccinid-like (*Pustulatirus mediamericanus, Opeatostoma pseudodon*) to fasciolariid–like (*Fasciolaria lignaria*). The salivary ducts, when it was possible to follow them, passed freely along the esophagus, or were bound with it by connective tissue, but in no case were embedded into the esophagus walls. A single morphological character was common to all fasciolariids and was never found in buccinids. This character is the structure of the proboscis retractor muscles, represented in fasciolariids by single or paired tufts of muscle fibers. In contrast, all buccinids studied have retractor muscles consisting of multiple muscle tufts, sometimes packed into two secondary tufts by connective tissue (Kosyan and Kantor, 2009). Thus, from a morphological perspective, Fasciolariidae constitute a derived group within Buccinidae. It is noteworthy that this pattern also emerged from our preliminary molecular analysis.

In the phylogenetic hypothesis derived from the molecular dataset (Figure 30), including 16 buccinid and 4 fasciolariid taxa, *Troschelia* is in the same clade with the tropical buccinids *Pareuthria* and *Phos*. Although the relationships among several buccinid clades are still not clearly resolved in our topology (possibly due to both a significantly incomplete taxonomic coverage, and the use of a suboptimal marker), we recovered a strong signal of close relationship between Fasciolariidae and Buccinidae.

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Coralliophilinae (Gastropoda: Muricidae) associated with deep-water coral banks in the Mediterranean

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ABSTRACT

Fisheries and scientific investigations of the recently discovered deep-water coral province south of Malta sampled living specimens of two deep-water Coralliophilinae intimately associated with *Lophelia-Madrepora* coral banks. The species are *"Coralliophila" richardi* (Fischer P., 1882) and *Babelomurex sentix* (Bayer, 1971). A third coralliophilinid *"Coralliophila" squamosa* (Bivona Ant. *in* Bivona And., 1838: deep-water morphotype) has been also observed alive close to deep-water corals at the Nameless-Urania Bank.

Additional keywords: Neogastropoda, cnidaria, predation, biogeography, amphi-Atlantic, biodiversity

INTRODUCTION

Deep-water coral ecosystems are receiving increasing attention from the scientific community as biodiversity hotspots (Freiwald et al., 2004; Roberts et al., 2006). The Mediterranean Sea hosts a variety of deep-water corals inhabiting soft and hard substrates. Some skeletonized cnidarians (mostly the scleractinians *Lophelia pertusa* (Linnaeus, 1758), *Madrepora oculata* Linnaeus, 1758; *Desmophyllum dianthus* (Esper, 1794), *Javania cailleti* (Duchassaing and Michelotti, 1864), *Caryophyllia* spp., *Dendrophyllia* spp., the gorgoniacean *Corallium rubrum* (Linnaeus, 1758), and several others) may contribute to the formation of considerable bioconstructions at depths in excess of 300 m (Taviani et al., 2005; Freiwald et al., 2009). Such living deep-water coral assemblages are widespread in the Mediterranean basin as are still-submerged taphocoenoses and outcrops (Taviani et al., 2005).

Unravelling the interactions between cnidarians and their predators is essential for a better understanding of the ecology of deep-water coral banks. Top predators of cnidarians include gastropods belonging to the families Ovulidae, Epitoniidae, Janthinidae, Muricidae-Coralliophilinae, and Architectonicidae (Graham, 1965; Oliverio, 1989; Bieler & Petit, 2005; Schiaparelli et al., 2005; Gittenberger, 2006, with references). However, there are few documented reports of gastropod predation on Mediterranean deep-water corals due to: (1) the relative paucity of deep-water corals living in this basin, (2) the rarity of most coral-associated gastropod taxa, and (3) the inherent difficulties in imaging or sampling these deep-water habitats.

Maltese, Italian, and German oceanographic cruises (Figure 1), sampled three rare deep-water Coralliophilinae at deep-water coral (dwc) sites in the Strait of Sicily: *"Coralliophila" richardi* (Fischer P., 1882), *Babelomurex sentix* (Bayer, 1971), and *"Coralliophila" squamosa* (Bivona Ant. in Bivona And., 1838: morphotype better known as *Pseudomurex ruderatus* Sturany, 1896) respectively. The present report documents these findings (Table 1).

CORALLIOPHILINES FROM MEDITERRANEAN DEEP-WATER CORAL SITES

"Coralliophila" richardi (Fischer P., 1882)

Murex richardi Fischer P., 1882: 49
Coralliophila lactuca Dall, 1889: 220, pl. 16, fig. 6
Coralliophila richardi.—Bouchet and Warén, 1985: 152, fig. 368

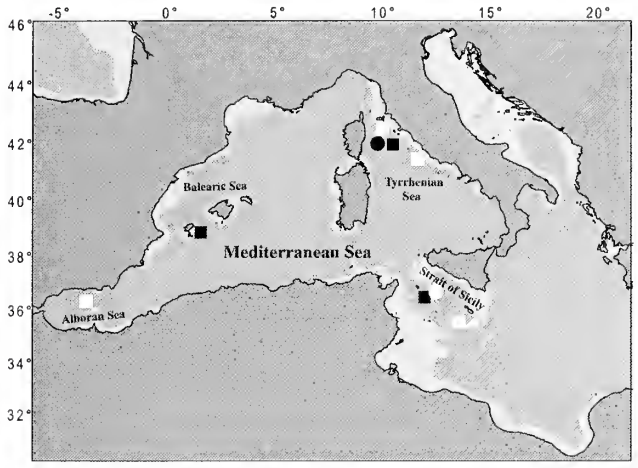


Figure 1. Map showing station localities discussed in this report. Symbols: □, live *Coralliophila richardi* (from literature and this paper); ■, subfossil *C. richardi* (from literature and this paper); △, *Babelomurex sentix*; ○, *'Coralliophila' squamosa* (morphotype *ruderatus*), ●, subfossil *'Coralliophila' squamosa* (morphotype *ruderatus*).

Remarks: Two living specimens of *'Coralliophila' richardi* (Figures 2–6) were trawled from *Lophelia-Madrepora* coral banks off Malta during the GRUND 2003 mission (see Schembri et al., 2007). Additional pre-modern material (Figures 7–9; most likely glacial Pleistocene fossils) was collected over many decades of sampling during the CNR-Bologna oceanographic missions of the research vessels BANNOCK (see Bouchet and Warén, 1985; Taviani and Taviani, 1986) and URANIA (this study).

Murex richardi (described from the Bay of Biscay) is the senior synonym of *Coralliophila lactuea* Dall, 1889 (from off Cuba and Fernandina, Florida in the Western Atlantic: Bouchet and Warén, 1985; Taviani and Taviani, 1986). This amphi-Atlantic species is now known from various sites in the eastern Atlantic Ocean (Rolan and Pedrosa, 1981; Oliverio and Gofas, 2006) and has been reported living in the Tyrrhenian and Alboran Seas (Cecalupo, 1984; Oliverio, 1989; Giusti, 1996; Giannuzzi-Savelli et al., 2003). It also occurs as an Early Pleistocene fossil in deep-water deposits of presumed Sicilian age in southern Italy (Vazzana, 1996).

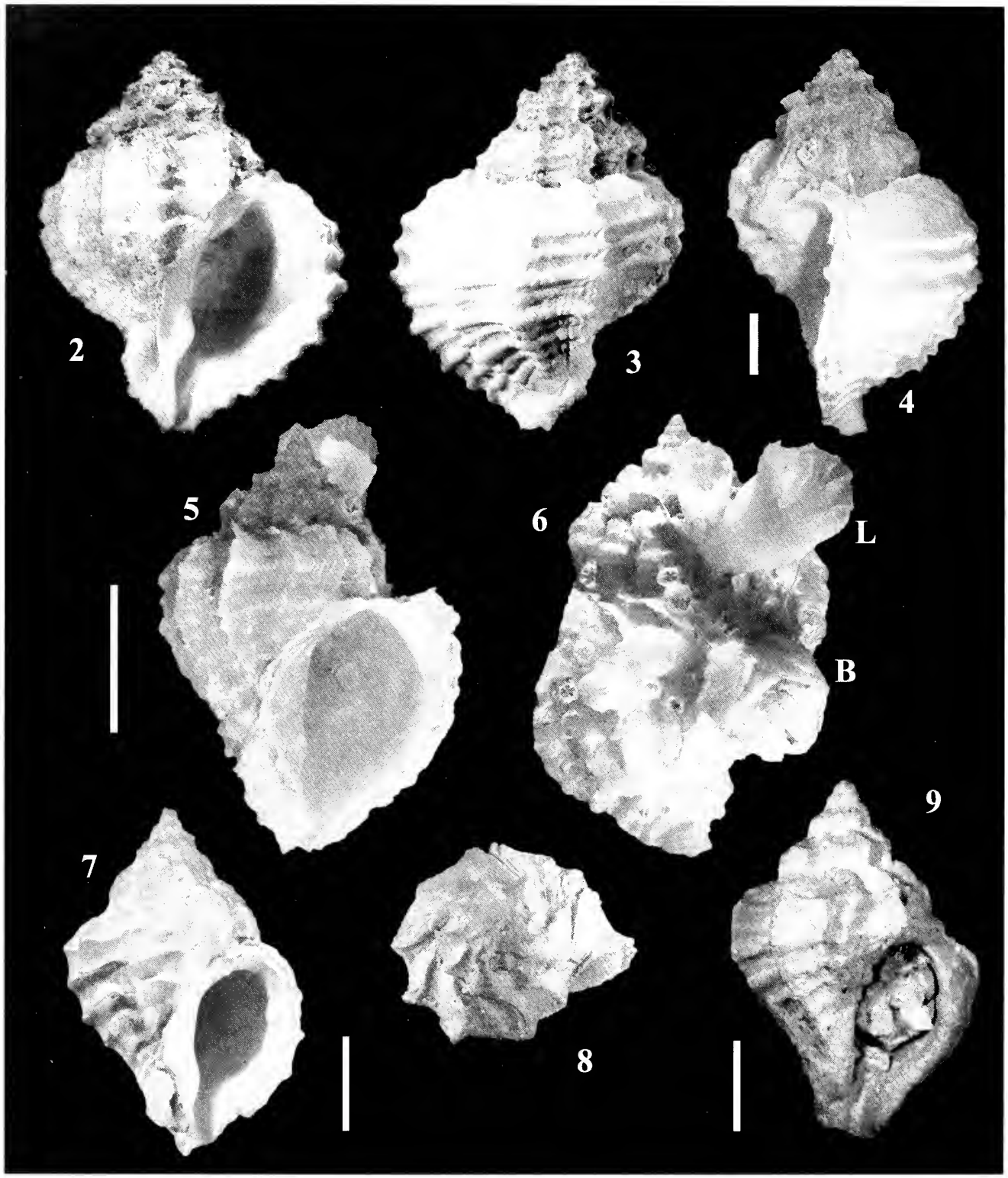
The taxonomic affinities of *"Coralliophila" richardi* are obscure. The shell morphology of this species is unusual within the subfamily Coralliophilinae, and is shared only with *Emozamia lieinus* (Hedley and Petterd, 1906), a deep-water, western Pacific species. Genetic studies of Mediterranean (this material) and Atlantic specimens will likely elucidate the taxonomy of this group.

The consistent co-occurence of *"Coralliophila" richardi* with the scleractinians *Lophelia* and *Madrepora* in Recent and pre-modern assemblages has led to the suggestion that this taxon is likely a predator of one or both corals (e.g., Taviani and Colantoni, 1979). The regularly arched shape and dimension of the shell aperture of *"C." richardi* seem well adapted for a sedentary position on a branching stony coral colony such as those of *Madrepora* or *Lophelia*.

This hypothesis is supported by the co-occurence of live *Lophelia, Madrepora,* and *"C." richardi* off Malta, the latter fouled by juvenile *Lophelia* corals (Figures 5–6). Information from Atlantic Ocean specimens further supports the hypothesis of a strict relationship between *C. richardi* and branching deep-water corals. A specimen was photographed still adhering to the surface of living *Madrepora* on the Galicia Bank (Figure 16) (42°48.37′ N, 11°47.47′ W, 880 m depth). *"Coralliophila" richardi* has also been reported from various seamounts in the eastern Atlantic (Oliverio and Gofas, 2006), where it co-occurs with living or dead coral (mostly *Madrepora*: S. Gofas, unpublished notes, and M.T., unpublished notes). In the western Atlantic, three live specimens of *C. richardi* were collected with living corals on a *Lophelia* lithoherm (peak # 160) off St. Augustine, Florida (29°50.9726′ N, 79°37.5976′ W, in 871–746 m, bottom temperature 7.96°C; salinity 35.1) during dive JSL-I-4912 (Chief Scientist J. Reed), 11 Nov. 2005.

Table 1. Main attributes of stations yielding the Mediterranean coralliophilines discussed in the text.

Cruise	Sample no.	Area	Start Long. N	Start Lat. E	Start Depth (m)	End Long N	End Lat E	End Depth (m)	Species
CS73	7	Nameless-Urania Bank	36°53.600′	13°06.300′	695	36°51.800′	13°06.300′	410	*Coralliophila richardi*
ET95	D21	Tuscan Archip.	43°18.850′	09°48.920′	582	43°19.450′	09°49.080′	515	*C. richardi*
GRUND2003	G19	Malta	35°30.47′	14°06.27′	617	35°30.830′	14°06.020′	420	*C. richardi*
MARCOS	MS43	Malta	35°30.720′	14°06.561′	607	35°30.803′	14°06.511′	452	*Babelomurex sentix*
MARCOS	MS44	Malta	35°30.506′	14°06.230′	632	35°31.228′	14°05.698′	467	*B. sentix*
CORTI	CORTI71	Tuscan Archip.	43°13.505′	09°36.326′	369	43°13.682′	09°36.260′	399	*Coralliophila squamosa* (morphotype *ruderatus*)
M70-1	677	Nameless-Urania Bank	36°50.340′	13°09.300′	544	36°50.340′	13°09.390′	388	*C. squamosa* (morphotype *ruderatus*)



Figures 2–9. *Coralliophila richardi*. **2–6.** Living *Coralliophila richardi* from Malta coral banks (st. GRUND 2003-G19). **2–4.** Simons outer lip accommodates settlement on coral branch. Scale bar = 1 cm. **5–6.** Fouling by scleractinian corals (e.g., *Lophelia pertusa*: A. Vertino, pers. comm., 2008) and barnacles. Scale bar = 1 cm. **7–9.** Specimens from Pleistocene submerged assemblages. **7–8.** Strait of Sicily (Station CS73-7). Scale bar = 1 cm. **9.** Tuscan Archipelago (Station ET95-D21). Scale bar = 1 cm.

"Corallioplula" richardi also occurs in the Gulf of Mexico on live deep-water coral banks. Norem et al. (2008: pl. 27B) illustrated two specimens of *"C." richardi* (identified as the shallow-water *"C." abbreviata* (Lamarck, 1816)), on live coral from the *Lophelia* banks of the Viosca Knoll in circa 315 m depth (dive JSL 4747).

Babelomurex sentix (Bayer, 1971)

Corallioplula sentix Bayer, 1971: 189, fig. 49
Latiaxis sentix carcassii Nicolay and Angioy, 1985: 16–18

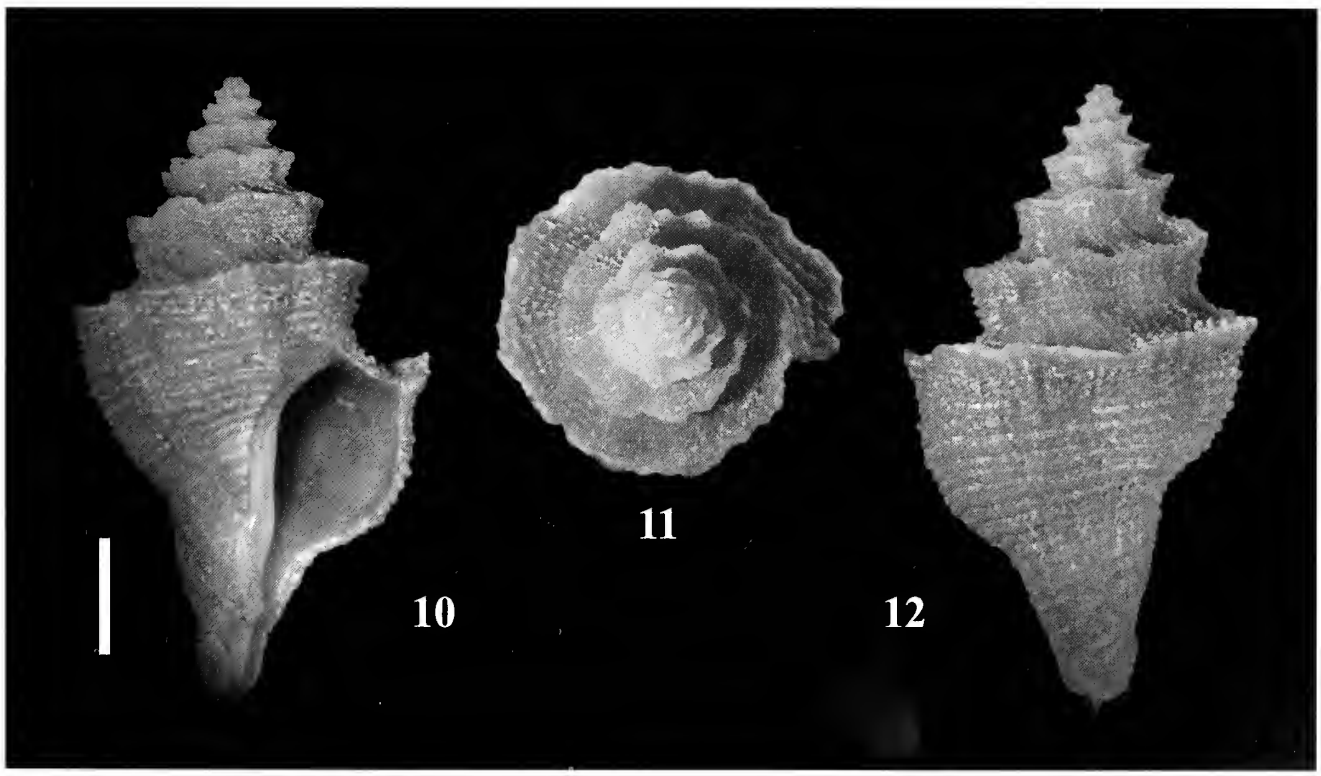
Remarks: *Babelomurex sentix* (originally described from east of St. Vincent, Lesser Antilles) is a rare amphi-Atlantic species seldom found alive (Bayer, 1971; Oliverio and Gofas, 2006). There are a few scattered records from the western basin of the Mediterranean Sea off Sardinia, Melilla, and Alboran. Within this basin, fresh shells, including some with operculum, document that this species has been found alive in the Mediterranean more than once (Nikolay and Angioy, 1985: as *Latiaxis sentix carcassii*; Oliverio, 1989; Giannuzzi-Savelli et al., 2003).

Two living specimens (Figures 10–12) and one shell of *Babelomurex sentix* were trawled from south of Malta from coral banks dominated by adult *Lophelia, Madrepora,* and *Desmophyllum* and small colonies of *Corallium* in 2007 during the MARCOS cruise (Chief Scientist Marco Taviani). The animals were kept alive in the aquarium onboard the ship for a week and were quite active, thus permitting a full documentation of their expanded soft parts (Figures 18–20). Its presumed association with white corals (Oliverio, 1989) is only based on indirect evidence.

"Corallioplula" squamosa (Bivona Ant. in Bivona And., 1838)

Fusus squamosus Bivona Ant. in Bivona And., 1838: 14; fig. 22
Murex alucoides Blainville, 1829: 128; pl. 5B fig. 1 (non *Murex alucoides* Olivi 1792)
Fusus lamellosus Philippi, 1836 [ex de Cristofori and Jan ms.]: 204–205, pl. 11 fig. 30 (non *Fusus lamellosus* Borson, 1821)
Fusus squamulosus Philippi, 1836: 204, pl. 11 fig. 31 (non *Fusus squamulosus* Deshayes, 1835)
? Pseudomurex perfectus Fischer P., 1883: 274
? Pseudomurex ruderatus Sturany, 1896 [ex Monterosato ms.]; 26, pl. 2 fig. 42–43
? Pseudomurex monterosatoi Locard, 1897: 315, pl. 15 fig. 21–23

Remarks: *"Corallioplula" squamosa* (originally described from Sicily, but currently with a neotype from Corsica: Bouchet and Waren, 1985), is a relatively common and widespread taxon known throughout the Mediterranean Sea. It is presumed to be associated with gorgonians, and, on the deeper continental shelf, with scleractinians (Oliverio, 1989), although there is no direct evidence for this.



Figures 10–12. Living specimens of *Babelomurex sentix* collected from Malta deep-water coral banks during the MARCOS cruise (Station MS43). Scale bar = 1 cm.

A plausible association of *"C." squamosa*, recorded as larger and smoother morphotypes of *"Coralliophila" lamellosa* (de Cristofori and Jan, 1832), with Mediterranean deep-water corals was reported by Taviani and Colantoni (1979). These shells are included in *Pseudomurex ruderatus* (Sturany, 1896). *Pseudomurex ruderatus* may represent a deep-water morphotype of the variable Atlantic-Mediterranean *"Coralliophila" squamosa* and their mutual relationships will be elucidated by an on-going genetic study.

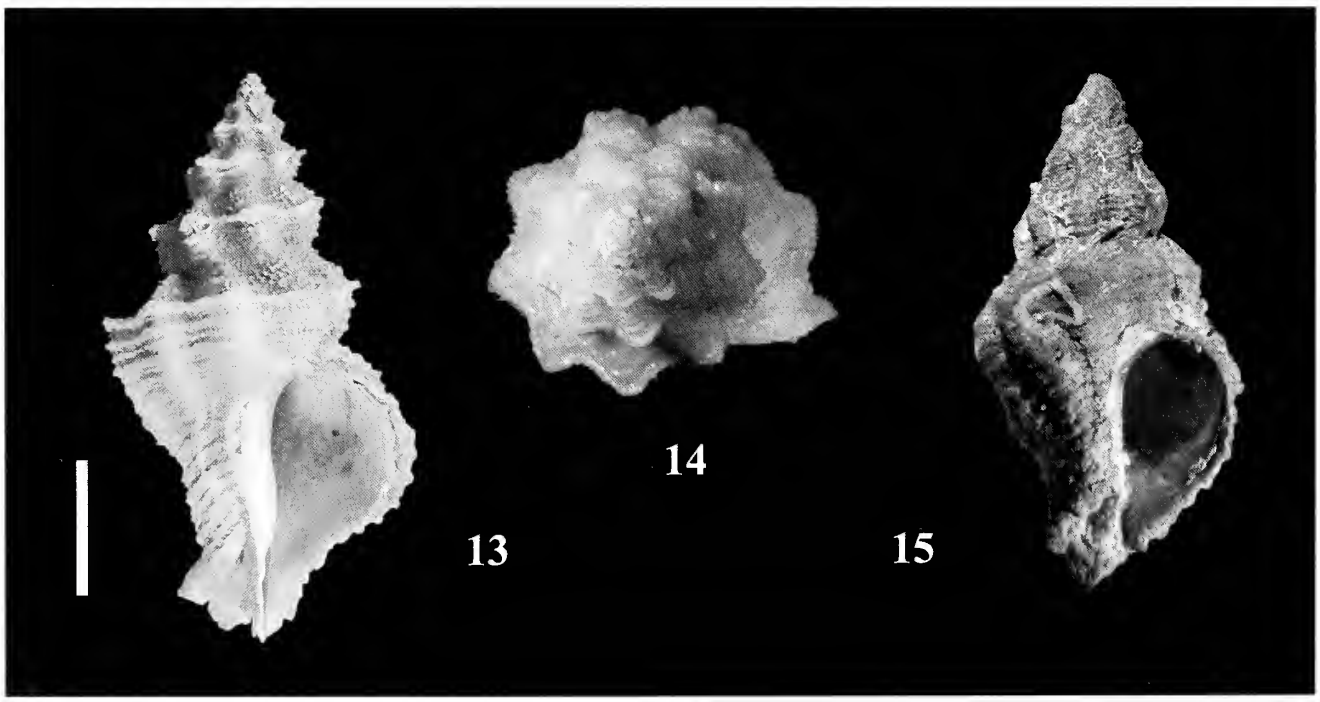
A single live individual of *"Coralliophila" squamosa* (morphotype *ruderatus*: Figures 13–14) has been photographed and then collected using the MARUM ROV Quest 4000 m during cruise M70-1 of R/V Meteor (Chief Scientist A. Freiwald). A single living specimen (Figure 17) was found on the volcanic bedrock at circa 500 m off the Nameless-Urania Bank, Strait of Sicily. The ROV images document a variety of co-occurring cnidarians at this site including *Lophelia*, *Madrepora*, *Desmophyllum*, *Corallium*, as well as antipatharians and gorgonians. Other empty shells collected from various deep-water sites in the Mediterranean basin may also belong to this elusive taxon (Figure 15).

CONCLUSIONS

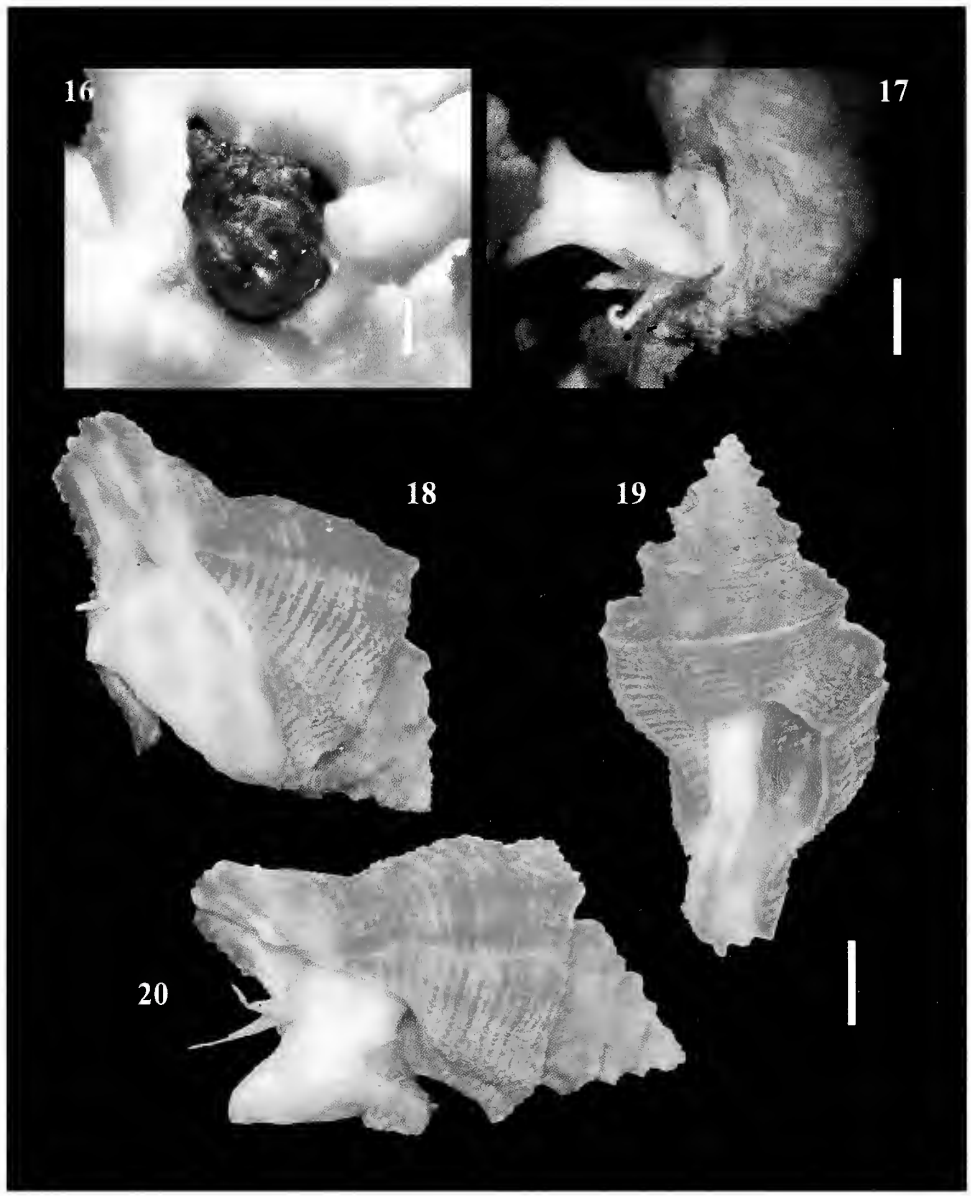
Of the coralliophilines associated with deep-water coral banks, *"Coralliophila" richardi* is strictly associated with *Lophelia* and very likely with *Madrepora*. *Babelomurex sentix* and *"C." squamosa* (morphotype *ruderatus*) seem confined to deep water coral banks, but their precise hosts have yet to be identified. The supraspecific position of these three bathyal coralliophilines is still unclear.

Recent collections of living specimens of these rare Coralliophilinae have provided material for molecular systematic studies, as well as for determination of their host cnidarians through DNA barcoding of their gut contents (Oliverio and Mariottini, 2001; Oliverio et al., 2009).

The biogeography of these very rare coralliophilines merits attention. For all three species, connections between Mediterranean and Atlantic populations may be linked to their supposedly teleplanic larvae. All three species (*"C." squamosa*, *"C." richardi*, and *B. sentix*) have established populations in the Atlantic Ocean. Their planktotrophic larvae may have been passively dispersed into the Mediterranean by currents. This may have led to the establishment of viable populations in this basin (as it is certainly the case for *"C." squamosa*) although the possibility of non-reproductive pseudo-populations (Bouchet and Taviani, 1992) can not be ruled out. However, *"Coralliophila" squamosa* is not uncommon (with its typical morphotypes) in shallower waters throughout its range, and the rarity of its putative deep-water morphotype *ruderatus* may be related to sampling difficulties. *"Coralliophila" richardi* is known from multiple sites in the western Mediterranean and this suggests a status of permanent resident in the basin, also supported by its prolonged, albeit not necessarily continuous, presence in this basin since the Early Pleistocene.



Figures 13–15. *"Coralliophila" squamosa* (morphotype *ruderatus*). **13–14.** Live-collected specimen from the Nameless-Urania Bank, Strait of Sicily, Station M70/1-677. **15.** Shell from a Pleistocene submerged assemblage, Tuscan Archipelago, Station CORTI-71. Scale bar = 1 cm.



Figures 16–20. Living Coralliophilinae. **16.** *"Coralliophila" richardi* on living *Madrepora oculata*, Galicia Bank. Scale bar = 3 mm. **17.** *In situ* photograph of *'Coralliophila' squamosa* (morphotype *ruderatus*) recovered from the Nameless-Urania Bank (Station. M70/1-677). Scale bar = 5 mm. **18–20.** *Babelomurex sentix* with extended soft parts, collected during the MARCOS cruise. **18, 20.** Adult specimen from Station MS44. **19.** Immature specimen from Station MS43. Scale bar = 1 cm.

Records of *B. sentix* in the Mediterranean Sea are scanty. Further evidenee is the needed for us to demonstrate the presence of permanent populations in the region.

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The coralliophiline (Gastropoda: Muricidae) radiation: repeated colonizations of the deep sea?

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ABSTRACT

The Coralliophilinae are a subfamily of Muricidae, with about 200–250 species, mostly from temperate and tropical oceans, that are associated with anthozoans on which they feed. We present here a phylogenetic hypothesis for the subfamily, based on DNA sequences (650 aligned positions) of the mitochondrial 12S rDNA from 42 coralliophilines and six other muricids, as well as one fasciolariid, which serves as the outgroup. Relationships among the muricid subfamilies were not resolved unequivocally, but coralliophiline monophyly was strongly supported. Two major clades emerged within the Coralliophilinae, both well supported in a Bayesian analysis. The genera *Coralliophila* and *Babelomurex* as commonly understood, are clearly polyphyletic, and in need of redefinition. Our results indicate multiple, independent incursions of Coralliophilinae into deep water habitats, several producing subsequent radiations.

Additional keywords: Neogastropoda, Coralliophilinae, *Coralliophila*, *Babelomurex*, 12s rDNA

INTRODUCTION

Coralliophila and related genera (e.g., *Babelomurex*, *Latiaxis*, *Leptoconchus*, and *Quoyula*) comprise the muricid subfamily Coralliophilinae, a highly diverse lineage of neogastropods that contains approximately 200–250 described species distributed worldwide, mostly in warm temperate and tropical oceans. These species are traditionally partitioned among 7–10 genera based on shell morphology. The known fossil record for coralliophilines extends to at least the middle Eocene (circa 40 Ma). All species for which the ecology is known are symbionts (ecto or endobiotic) of anthozoans (including sea-anemones, gorgonians and reef-building coral species), on which they feed.

The Coralliophilinae are well represented in deep water faunas of the tropical and subtropical Atlantic and Indo-Pacific oceans. Deep habitats are those in excess of 100–150 m, which is the depth limit for hermatypic scleractinians. Beyond these depths, they are replaced by Alcyonaria, Stylasterina and Porifera. In temperate regions, these depth limits are likely to be closer to the surface, in the range of 50–100 m (mostly dependant on the turbidity of the water), where marine phanerogams and green algae (along with the non hermatypic zooxanthellate hexacorals) are progressively replaced by sponges, red algae, and octocorals.

A detailed, quantitative analysis of shallow and deep faunas by area has not yet been conducted. However, even a conservative approach (i.e., extrapolating the "tropical" bathymetric boundary of 100–150 m to all regions) reveals a high proportion of deep-water species within Coralliophilinae, ranging between 65–80% for most regions, with a global average of 75% (Oliverio, 2008a, 2008b, In press; Marshall and Oliverio, In press; M. Oliverio and C. Smriglio, pers. observ.)

In the absence of a detailed fossil record of the Coralliophilinae, it is unclear whether the group originated in shallow water, with subsequent colonizations of deep water habitats, or if the ancestral members of the subfamily evolved in deeper waters, with subsequent invasions of photic habitats. A phylogenetic framework would aid in distinguishing between these mutually exclusive evolutionary scenarios.

Previous studies based on the morphology of digestive and reproductive systems, along with data on developmental and alimentary ecology (Richter and Luque, 2002), indicated monophyly of coralliophilines, with significant differences from the muricid *bauplan*, suggesting a derived, monophyletic radiation from an early muricoidean ancestor. Preliminary molecular phylogenetic studies (Oliverio and Mariottini, 2001; Oliverio et al., 2002),

despite limited taxonomical coverage (11 species, ~5% of the known species), clearly showed that coralliophilines originated within the muricid radiation, indicating a probable sister group relationship with the rapanine lineage(s).

We present herein a phylogenetic study based on partial sequence of the 12S rDNA (a portion corresponding to domain III), performed on 35 coralliophiline species (i.e., circa 15% of their known species diversity) and seven outgroup taxa. The goal of this study is to uncover the relationships of the Coralliophilinae within the family Muricidae, and of as many genera as possible within the subfamily.

MATERIALS AND METHODS

Taxon Sampling and Specimen Collection: A total of 41 sequences were analyzed in this study. Of these, 12 were derived from previous works (Oliverio and Mariottini, 2001; Oliverio et al., 2002; Mariottini et al., 2005). Thirty new sequences were determined with the goal of enlarging the taxonomic coverage to include as much of the morphological diversity of the Coralliophilinae as possible. Taxon names, locality data, voucher information, and EMBL (The European Molecular Biology Laboratory, Heidelberg) accession numbers are provided in Table 1. *Fasciolaria lignaria* (Fasciolariidae) was selected to serve as the outgroup for our sequence analyses. Voucher specimens of most samples are stored at Muséum national d'Histoire naturelle (MNHN, Paris) and at Dipartimento di Biologia Animale e dell'Uomo (DBAU, Rome). Double ID in Table 1 indicates that the primary voucher is stored at MNHN and tissue samples of the voucher and/or specimen(s) from the same lot are stored at DBAU.

Sequences from six muricids, representing five additional subfamilies were included in our analyses in order to reassess the monophyly of Coralliophilinae, and the sister group relationship with the Rapaninae that was previously hypothesized by Oliverio and Mariottini (2001).

DNA Extraction, PCR, Cloning and Sequencing: Total DNA was extracted following a standard Phenol/Chloroform/Ethanol protocol (Hillis et al., 1990) with slight modification as previously described by Oliverio and Mariottini (2001). DNA from difficult samples was extracted by the QIAGEN QiAmp Extraction Kit, according to manufacturer's instructions. DNA from formalin-fixed samples was extracted with the standard protocol after washing the tissue sample 3–5 times with PBS.

Partial sequences of the mitochondrial gene encoding the 12S ribosomal DNA were PCR amplified, with the primers *12SI* (5′-TGCCAGCAGCCGCGGTTA-3′) and *12SIII* (5′-GAGCGACGGGCGRTTWGTAC-3′) (Oliverio and Mariottini 2001). Amplification conditions were as follows: 94°C for 30 seconds, 45–50°C for 30 seconds, 72°C for 60 seconds (30–35 cycles). The PCR products were purified using the Exo-Sap enzymatic method, and double strand sequenced using the PCR primers. Sequencing was performed by Macrogen Inc. (Seoul, Korea). Chromatograms were analysed by Staden Package (Version 1.6.0, Staden et al., 1998, 2005). All sequences have been deposited at EMBL (see Table 1 for accession numbers).

Sequence and Phylogenetic Analysis: The 12S sequences were aligned using the default settings in ClustalX (Thompson et al., 1997) and then manually edited. Sequence data were analyzed for their fit (AIC criterion) to different models of nucleotide substitution using Modeltest v. 3.7 (Posada and Crandall, 1998) and MrModeltest v. 2.2 (Nylander, 2004) with the package PAUP* v. 4.0b10 (Swofford, 2002).

Analysis of the nucleotide sequence was performed using Mega3.1 (Kumar et al., 2004). The uncorrected pairwise distances (p) and the ML distances (i.e., pairwise distances corrected by the assumed model of evolution estimated) between the sequences were calculated. To test for the presence of mutational saturation, uncorrected p distances, transition (Ts) and transversion (Tv) were plotted against the estimated ML distance (Nichols, 2005; Philippe et al., 1994). The aligned sequences were analyzed under the assumptions of maximum likelihood (ML: Felsenstein, 1981) and by Bayesian inferences (BI), using the packages Treefinder (Jobb, 2007) and MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003), respectively. Support to the nodes was calculated for ML trees by using the Expected-Likelihood Weights (ELW: Strimmer and Rambaut, 2002) and bootstrap (bs) for 1000 replicates, as computed in Treefinder. A Bayesian analysis (BI) was performed to obtain posterior probabilities of branches using the software MrBayes, which adopts the Markov Chain Monte Carlo method to sample from posterior densities (Larget and Simon, 1999; Yang and Rannala, 1997). The model of evolution was the one chosen by MrModeltest. A four chain metropolis-coupled Monte Carlo analysis was run twice in parallel for 1.5×10^6 generations, and trees were sampled every 100 generations, starting after a burn-in of 375,000 generations. Bayesian posterior probabilities (bpp) were estimated on a 50% majority rule consensus tree of the sampled trees (after burn-in).

RESULTS

Partial sequences of the 12S ribosomal rRNA genes were determined and analyzed to explore the phylogenetic relationships among coralliophilines representing 34 species in 7 genera. The resulting sequences ranged in length from 507 bp in *Coralliophila panormitana* (Monterosato, 1869) to 548 bp in *Hexaplex trunculus* (Linnaeus, 1758) excluding the primers. The multiple sequence alignment resulted in a total of 563 nucleotide positions, including gaps.

Modeltest and MrModeltest estimated the GTR+I+G model (α = 0.7948; Pinvar = 0.2336) as the relatively best-fit model of nucleotide substitution for the dataset. The mutational saturation analysis (not shown) indicated that transitions started becoming saturated at a ML distance corresponding to inter-subfamilial comparisons.

Table 1. Species included in the molecular analysis, with voucher ID (BAU: Dept of Animal and Human Biology, Rome; MNHN, Muséum National d'Histoire Naturelle, Paris; NMSA: Natal Museum, Pietermaritzburg), collecting data, and EMBL accession numbers. OM2001 refers to Oliverio and Mariottini (2001), MSR2005 to Mariottini, Smriglio and Rolán (2005). If two IDs are given, the primary voucher is stored at MNHN (see text).

				EMBL Accession Numbers	
Family	Subfamily	Specimen ID	Locality	12S	Ref.
Muricidae					
Coralliophilinae	*Babelomurex amaliae* (Kobelt, 1907)	BAU00332	Astypalaya Is. (Greece) 36°33' N, 026°22' E, 35 m depth	AJ293671	OM2001
	Babelomurex armatus (Sowerby, 1912)	MNHN IM-2009-5110 BAU00333	Balicasag Is. (Philippines), tangle net. 9°30'50" N 123°41'16" E, 150 m depth.	FN391955	This work
	Babelomurex carinifernis (G. B. Sowerby II, 1834)	MNHN IM-2009-5111 BAU00334	Ustica Is. (Sicily, Italy), 10 m depth	FN391956	This work
	Babelomurex bernardi Nicolay, 1984	MNHN IM-2009-5112 BAU00335	C d'Ivoire, intertidal.	FN391957	This work
	Babelomurex cristatus (Kosuge, 1979)	MNHN IM-2009-5094 BAU00004	Panglao Is., Momo Beach (Philippines), PANGLAO 2004, st. P4. 9°36' N, 123°45' E, 80 m depth.	FN391958	This work
	Babelomurex deburghiae (Reeve, 1857)	MNHN IM-2009-5095 BAU00002	Bohol Island, Maribohoc Bay (Philippines), PANGLAO 2004, st. P1, 9°36' S, 123°45' E, 90–200 m depth.	FN391959	This work
	Babelomurex diadema (A. Adams, 1854)	MNHN IM-2009-5096 BAU00003	Bohol Island, Maribohoc Bay (Philippines), PANGLAO 2004, st. P1, 9°36' S, 123°45' E, 90–200 m depth.	FN391960	This work
	Babelomurex gemmatus (Shikama, 1966)	MNHN IM-2009-5097 BAU00013	Panglao Is., Momo Beach (Philippines), PANGLAO 2004, st. P4. 9°36' N, 123°45' E, 80 m depth.	FN391961	This work
	Babelomurex lischkeanus (Dunker, 1822)	MNHN IM-2009-5098 BAU00010	Nord Bellona (New Caledonia), EBISCO 2005, st. DW2578, 20°21' S, 158°40' E, 440–505 m depth.	FN391962	This work
	Babelomurex nakayasui (Shikama, 1970)	MNHN IM-2009-5099 BAU00005	Bohol Island, Maribohoc Bay (Philippines), PANGLAO 2004, st. P1, 9°36' S, 123°45' E, 90–200 m depth.	FN391963	This work
	Babelomurex princeps (Melvill, 1912)	MNHN IM-2009-5100 BAU00355	Norfolk Ridge (New Caledonia), NORFOLK 1, st. CP1713, 23°22' S, 168°02' E, 204–216 m depth.	FN391964	This work
	Babelomurex spinosus (Hirase, 1908)	MNHN IM-2009-5101 BAU00006	Banc Kelso (New Caledonia). EBISCO 2005, st. DW2520, 24°06' S, 159°41' E, 350–400 m depth.	FN391965	This work
	Babelomurex yamatoensis Kosuge, 1986	MNHN IM-2009-5102 BAU00011	Banc Nova nord (New Caledonia) EBISCO 2005, st. DW2533, 22°18' S, 159°28' E, 360 m depth.	FN391966	This work
	Babelomurex yumimarumai Kosuge, 1985	MNHN IM-2009-5103 BAU00336	Scorff passage (Vanuatu), SANTO 2006, st. EP12, 15°32' S, 167°15' E, 97 m depth.	FN391967	This work
	Coralliophila brevis (Blainville, 1832)	BAU00337	La Maddalena Is. (Sardinia, Italy), 41°15' N, 009°26' E, 30 m depth	AJ293676	OM2001
	Coralliophila bulbiformis (Conrad, 1837)	MNHN IM-2009-5104 BAU00012	Baldwin Bay (Vanuatu), SANTO 2006, st. FR58, 15°35'S, 167°02'E, 3–18 m depth	FN391968	This work

(Continued)

Table 1. (Continued.)

Family	Subfamily	Specimen ID	Locality	EMBL Accession Numbers 12S	Ref.
	Coralliophila caribaea	BAU00338	Juan Dolio, Santo Domingo, 15 m depth	AJ293677	OM2001
	Coralliophila clathrata (A. Adams, 1854)	BAU00339	Mtwalume, Natal (South Africa) intertidal rock pools (D. Herbert leg, 14.iii.1986)	FN391969	This work
	Coralliophila costularis (Lamarck, 1816)	MNHN IM-2009-5113 BAU00340	Darsa Is., N side, Soqotra Archipelago (Yemen), 7 m depth, 9.ii.2000	FN391970	This work
	Coralliophila crosa (Röding, 1798)	MNHN IM-2009-5105 BAU00341	East Malo Island (Vanuatu), SANTO 2006, st. DB86, 15°38'S, 167°15'E, 13 m depth	FN391971	This work
	Coralliophila fontanangioyi Smriglio and Mariottini, 2000	MNHN IM-2009-5114 BAU00342	Teno Sur, Tenerife, Canary Is. (Spain), 28°20'30" N 16°55'30" W, 15 m depth 20.11.94 on *Madracis asperula*	FN391972	This work
	Coralliophila kaofitorum Vega-Luz, Vega-Luz and Luque, 2002	MNHN IM-2009-5115 BAU00343	Teno Sur, Tenerife, Canary Is. (Spain), 28°20'30" N 16°55'30" W, 32 m depth 20.11.94 on *Antipates wollastoni*	FN391973	This work
	Coralliophila meyendorffii (Calcara, 1845)	BAU00344	Cape Circeo (Latium, Italy), 41°11' N, 013°04' E, 7 m depth	AJ297519	OM2001
	Coralliophila violacea (Kiener, 1836)	BAU00345	Taiwan, 23°10'N, 120°05'E 5 m depth	AJ293679	OM2001
	Coralliophila panormitana Monterosato, 1869	BAU00346	Cape Circeo (Latium, Italy), 41°11' N, 013°04' E, 70 m depth	AJ293681	OM2001
	Coralliophila radula (A. Adams, 1855)	BAU00015	Rarotonga (Cook Islands), 21°12'S 159°43'W, 12 m depth.	FN391974	This work
	Coralliophila squamosissima (E. A. Smith, 1876)	NMSA D9663	Botcler Point, Zululand (South Africa). 27°00'42" S 32°52'00" E, intertidal rock pools, amongst *Palythoa* sp. July 1987	FN391975	This work
	Coralliophila trigoi Mariottini, Smriglio, and Rolán, 2005	BAU00347	Camarinas, Galicia (Spain), northeastern Atlantic Ocean, 15–50 m depth	AJ937305	MSR2005
	Hirtomurex filiaregis (Kurohara, 1959)	MNHN IM-2009-5106 BAU00014	West Bellona (New Caledonia) EBISCO 2005 st. DW2549, 21°07 ' S, 15S°38' E, 330 m depth.	FN391976	This work
	Latiaxis hayashii (Shikama, 1966)	MNHN IM-2009-5116 BAU00348	Norfolk Ridge, 23°45'S, 168°17'E, 400–500 m depth.	FN391977	This work
	Latiaxis pilsbryi Hirase, 1908	MNHN IM-2009-5107 BAU00008	Banc Nova north (New Caledonia) EBISCO 2005, st. DW2534, 22°17' S, 159°2S' E, 390 m depth.	FN391978	This work
	Leptoconchus sp.	MNHN IM-2009-5108 BAU00062	Panglao Is., Sungcolan (Philippines), PANGLAO 2004, st. R47, 9°39' S, 123°49' E, 4–25 m depth.	FN391979	This work
	Quoyula monodonta (Sowerby, 1832)	BAU00349	Bunaken Is. (Sulawesi, Indonesia), 01°37'N, 124°46'E.	AJ293675	OM2001

(Continued)

Table 1. (Continued.)

Family	Subfamily	Specimen ID	Locality	EMBL Accession Numbers 12S	Ref.
	Rapa rapa (Linnaeus, 1758)	MNHN IM-2009-5109 BAU00085	Panglao Island, Napaling (Philippines), PANGLAO 2004, st. R19, 9°37' N, 123°46' E, 2–54 m depth.	FN391980	This work
Ocenebrinae	*Nucella lapillus* Linnaeus, 1758	BAU00187	Portobello (UK), 55°57' N 3°06' W, intertidal	AJ293668	OM2001
Muricinae	*Hexaplex trunculus* (Linnaeus, 1758)	BAU00351	San Pietro Is. (Sardinia, Italy), 39°09' N, 008°12' E, 3–4 m depth	AJ293669	OM2001
Muricopsinae	*Muricopsis cristata* (Brocchi, 1814)	MNHN IM–2009–5117 BAU00352	San Pietro Is. (Sardinia, Italy), 39°09' N, 008°12' E, 3–4 m depth	FN391981	This work
Rapaninae	*Cronia* sp.1	MNHN IM-2009-5118 BAU00619	Tolo Channel, Hong Kong, 22°27' N, 114°16' E, 1 m depth	FN391982	This work
	Cronia sp.2	MNHN IM-2009-5119 BAU00188	Rarotonga (Cook Islands), 21°12' S 159°43' W, 12 m	FN391983	This work
	Stramonita haemastoma (Linné, 1767)	BAU00354	San Pietro Is. (Sardinia, Italy), 39°09' N, 008°12' E, 3–4 m depth	AJ293670	OM2001
Fasciolariidae					
Fasciolariinae	*Fasciolaria lignaria* (Linnaeus, 1758)	BAU00350	San Pietro Is. (Sardinia, Italy), 39°09' N, 008°12' E, 3–4 m depth	AJ293682	OM2001

Figure 1 illustrates the tree recovered from the Bayesian analysis. As maximum likelihood ELW and bootstrap values (bs) were identical to four decimal places, only the bs are indicated on the tree, along with the bayesian posterior probabilities (bpp). Monophyly of Coralliophilinae was well supported in both ML (100 bs) and BI analyses (100 bpp). A sister-group relationship with the Rapaninae, represented in the tree by *Stramonita haemastoma*, did not receive high support, while BI supported a closer relationship of Coralliophilinae with Muricopsinae (represented by *Muricopsis cristata*) than with any other muricid.

The internal arrangement of the coralliophilines in the tree was characterized by the sorting of the species into two well-supported clades: Clade A (93 bpp) included *Quoyula monodonta*, *Babelomurex lischkeanus*, the endobiotic taxa (*Rapa*, *Leptoconchus*), and the Eastern Atlantic/Mediterranean species usually included in *Coralliophila;* Clade B (100 bpp, 100 bs) included the remaining *Coralliophila* species, along with the taxa traditionally ascribed to *Latiaxis*, *Hirtomurex*, and *Babelomurex*.

DISCUSSION

Coralliophilinae has been regarded (either explicitly or implicitly) as a monophyletic group since Thiele (1929), who erected the family Magilidae based on the absence of jaws or a radula. Subsequent workers suggested other characteristics (e.g., a long pleurembolic proboscis, absence of accessory salivary glands, absence of dorsal glandular folds of the oesophagus, and fusion of the paired salivary ducts into a single duct) as possible synapomorphies of Coralliophilinae (Gohar and Soliman, 1963; Ward, 1965; Ponder, 1973; Massin, 1987, 1990; Kantor, 1995). Our present molecular analysis, with a significant sampling of coralliophiline taxa for partial 12 S rDNA sequences, strongly supports the monophyly of Coralliophilinae (with high ELW, bootstrapped ML, and bpp), substantiating the preliminary conclusions from our previous studies (Oliverio and Mariottini, 2001; Oliverio et al., 2002).

Harasewych et al. (1997) as well as Oliverio and Mariottini (2001) and Oliverio et al. (2002), reported a sister group relationship between Coralliophilinae and Rapaninae. In the present study, we failed to recover this relationship. Instead, Muricopsinae and Ocenebrinae emerge as being more closely related to Coralliophilinae than did Rapaninae. However, given the level of saturation of transitions at maximum likelihood distances corresponding to inter-subfamilial comparisons, as well as the poor taxonomic sampling of muricid subfamilies, these results should be considered preliminary.

The results of the current analyses reveal that Coralliophilinae are resolved into two distinct clades (A and B in Figure 1), each with high levels of support, confirming the preliminary indications by Oliverio and Mariottini (2001) and Oliverio et al. (2002). Clade A includes *Quoyula monodonta*, *Coralliophila clathrata*, the endobiotic taxa (*Rapa*, *Leptoconchus*), the deep-water enigmatic taxon *"Babelomurex" lischkeanus*, and the Eastern

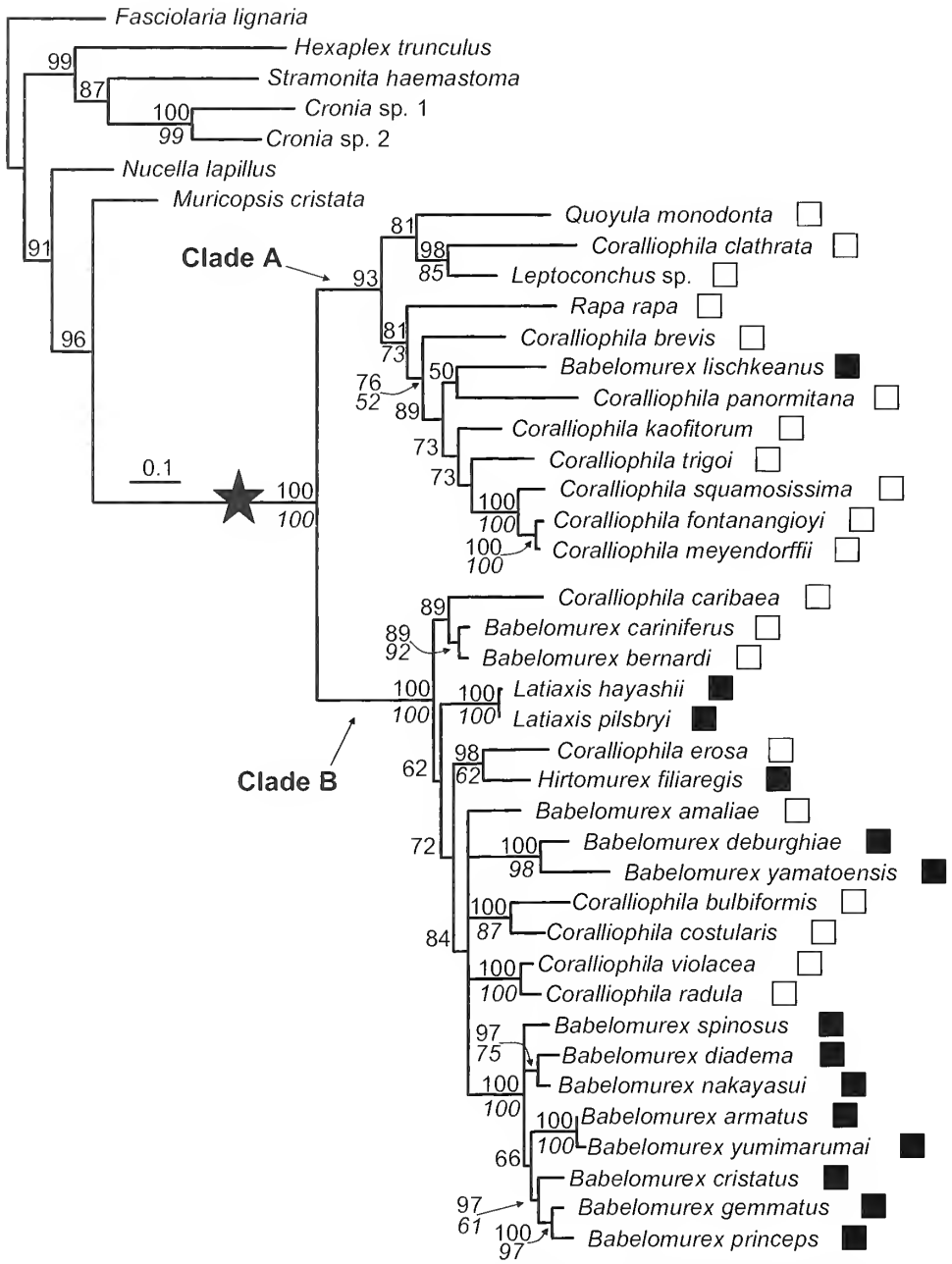

Figure 1. Bayesian tree, portraying phylogenetic relationships among the assayed species. Numbers at the node are Bayesian posterior probabilities (11,250 trees) and maximum likelihood bootstrap supports (1000 replicates; in *italic*). The star indicates the branch leading to the monophyletic Coralliophilinae. Symbols: ■, deep water species; □, shallow water species.

Atlantic/Mediterranean species usually included in *Coralliophila*. The positions of the endobiotic *Leptoconchus* sp. and *Rapa rapa* in the tree suggest that endobiosis may have originated at least twice in this group, a unparsimonious hypothesis to be further tested. *Leptoconchus* species are endoparasites of Hexacorallia, while *Rapa* species live within soft corals (Octocorallia). *Quoyula monodonta* feeds upon Scleractinia and *Coralliophila clathrata* on Zoanthidea. We infer that this clade had its origins as ectoparasites of shallow water hexacorals, with a single ascertained shift to octocorals (*Rapa*), two adaptations for endobiosis, and at least one colonization of deep water habitats ("*B.*" *lischkeanus*).

Clade B includes all the remaining coralliophiline species in our study. The close, sister group relationship (100 bs, 100 bpp) between *Latiaxis hayashii* and *L. pilsbryi* supports the monophyly of the genus *Latiaxis* sensu stricto (the type species, *L. mawae*, was not included in the analysis.) However, the monophylies of the widely used genera *Coralliophila* and *Babelomurex* are not supported.

Oliverio and Mariottini (2001) and Oliverio et al. (2002) suggested that the genus *Coralliophila*, as usually understood, may be polyphyletic. The type species of *Coralliophila*, *C. violacea*, forms a pair with the morphologically similar, Indo-Pacific *C. radula*, and, with another pair (*C. bulbifomis* and *C. costularis*), are included in a clade predominated by eleven species of *Babelomurex*. Two other species, the Caribbean *C. caribaea* and the Indo-Paeific *C. erosa*, belong in Clade B. Most of the speeies usually included in *Coralliophila* s.l. are in clade B. We urge the need for a re-definition of the genus *Coralliophila*, and for a restriction of its use. For the other species traditionally included in *Coralliophila* there is a long list of names, potential candidates for the other lineages (e.g., *Pseudomurex* Monterosato, 1872).

The type species of *Babelomurex*, the eastern Atlantic/Mediterranean *B. cariniferus*, emerges as the sister taxon of the West African *B. bernardi*, a relationship well supported by shell morphology. Both species live in shallow waters. Most of the remaining species assigned to the genus *Babelomurex* form a well defined clade (*B. spinosus*, *B. diadema*, *B. nakayasui*, *B. armatus*, *B. yumimarumai*, *B. cristatus*, *B. gemmatus* and *B. princeps*), of deep-water Indo-Pacific species that do not appear to be monophyletic with the type species of *Babelomurex*. This group is likely the result of an independent radiation following the shift to a deep-water habitat by a shallow water ancestor. Other species of *Babelomurex*, *B. deburghiae*, *B. yamatoensis*, and even *B. amaliae*, form an unresolved polytomy with this clade. In addition, speeies presently assigned to *Hirtomurex* and *Latiaxis* live in a deep water habitat, representing possible additional deep-water colonization/radiation events within Coralliophilinae.

Considering the global pattern of bathymetrie distribution (~75% of known coralliophiline species in deep waters), a likely hypothesis for the coralliophiline radiation involves multiple colonizations of deep-water habitats, with many or most resulting in adaptive radiations. Unfortunately, the very limited information on the host associations of deep water Coralliophilinae (e.g., Taviani et al., 2009) is an impediment to a clear understanding of the factors involved in such radiations.

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Extremely slow feeding in a tropical drilling ectoparasite, *Vitularia salebrosa* (King and Broderip, 1832) (Gastropoda: Muricidae), on molluscan hosts from Pacific Panama

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ABSTRACT

This study documents one of the slowest feeding behaviors ever recorded for a muricid gastropod in one of the most biotically rigorous regions on the planet. In Pacific Panama, *Vitularia salebrosa* attacks mollusks by drilling through their shells. The duration of attacks estimated by isotope sclerochronology of oyster shells collected during attacks in progress range from 90 to 230 days, while experimental observation of interactions documented one attack greater than 103 days. The prolonged nature of attacks suggests that *V. salebrosa* is best characterized as an ectoparasite than as a predator, which is the ancestral condition in the Muricidae. An ectoparasitic lifestyle is also evident in the unusual interaction traces of this species, which include foot scars, feeding tunnels and feeding tubes, specialized soft anatomy, and in the formation of male-female pairs, which is consistent with protandrous hermaphroditism, as is typical in sedentary gastropods. To delay death of its host, *V. salebrosa* targets renewable resources when feeding, such as blood and digestive glands. A congener, *Vitularia miliaris* from the Indo-Pacific, has an identical feeding biology. The origin and persistence of extremely slow feeding in the tropics challenges our present understanding of selective pressures influencing the evolution of muricid feeding behaviors and morphological adaptations. Previously, it has been suggested that faster feeding is advantageous because it permits predators to spend a greater proportion of time hiding in enemy-free refugia or to take additional prey, the energetic benefits of which could be translated into increased fecundity or defenses. The benefits of exceptionally slow feeding have received little consideration. In the microhabitat preferred by *V. salebrosa* (beneath boulders), it is possible that prolonged interactions with hosts decrease vulnerability to enemies by reducing the frequency of risky foraging events between feedings. Ectoparasitic feeding through tunnels by *V. salebrosa* may also reduce competitive interactions with kleptoparasites (e.g., crabs, snails) that steal food through the gaped valves of dead or dying hosts.

Additional keywords: *Vitularia miliaris*, ectoparasite, foot scar, feeding tube, sclerochronology

INTRODUCTION

Predatory species of the neogastropod family Muricidae generally attack prey by slowly drilling a hole through the wall of the prey's shell, a process that can take from several days to just over a week (Palmer, 1990; Dietl and Herbert, 2005; Peharda and Morton, 2006). During this time, muricids are left exposed and vulnerable to attacks from their own enemies and to theft of food by competitors attracted to the chemical scent of drilling or the injured prey (Paine, 1963; Morissette and Himmelman, 2000; Ishida, 2004). Thus, several authors have argued that natural selection should favor the evolution of offensive weapons and behaviors (e.g., edge drilling, kleptoparasitism, toxins, shell grinding) that accelerate or completely replace slower styles of attack (Vermeij and Carlson, 2000; Herbert, 2004; Dietl et al., 2004). Faster feeding allows animals to spend more time in enemy-free refugia or to take additional prey, the energetic benefits of which could be translated into increased reproduction or defenses (e.g., large size, thicker shell, speed, toxins, etc.). Selection for faster feeding should be particularly important in "biotically rigorous" environments, where predation and competition pressures are most intense (Dudley and Vermeij, 1978; Vermeij and Currey, 1980; Vermeij, 1987, 2004).

The present study focuses on the feeding ecology of *Vitularia salebrosa* (King and Broderip, 1832), a muricid that is relatively common in rocky intertidal habitats beneath boulders in Pacific Panama where it feeds on other Mollusks. We document the unexpected occurrence of one of the slowest feeding behaviors ever recorded for a muricid in one of the most biotically rigorous regions on the planet, the tropical Pacific. Our finding on the duration of attacks together with information on the feeding traces, specialized anatomy and reproductive behavior of *V. salebrosa* are consistent with

an ectoparasitic rather than a true predatory mode of life. We also compare and contrast alternative hypotheses to explain the environmental conditions surrounding the rare evolutionary transition between a temporary intimate predator-prey interaction to a persistent ectoparasite-host interaction.

MATERIALS AND METHODS

Study Area: Mollusks were collected from under boulders in the exposed rocky intertidal around Venado Island, in the Gulf of Panama, near Panama City, Panama (8°52′ N, 79°35′ W) in August 2005 and January 2006. This island is approximately 1.6 km offshore but accessible by foot during extreme low tides. Upwelling of cold, nutrient-rich water in late winter/early spring and freshwater runoff during the summer rainy season affect surface water conditions in this region, with average annual temperature and salinity in near-surface waters (top 20 m) of the Gulf of Panama ranging from 19.3 to 27.7°C and 29.3 to 34.3‰, respectively (Smayda 1965, 1966; Wyrtki, 1966, 1981; Geary et al., 1992). A more detailed description of the oceanographic and hydrographic regime of the Gulf of Panama is found in Bemis and Geary (1996). The dominant rock-encrusting macrofauna at Venado Island includes bryozoans and suspension-feeding mollusks, including the oysters *Pinctada mazatlanica* (Hanley, 1856), *Spondylus calcifer* Carpenter, 1857, *Chama* sp., and *Ostrea* cf. *fisheri* Dall, 1914, a vermetid gastropod *Tripsycha (Eualetes) tulipa* (Chenu, 1843 ex Rousseau, MS), and the calyptraeid gastropods *Crucibulum (Crucibulum) spinosum* (Sowerby, 1824) and *Bostrycapulus calyptraeformis* (Deshayes, 1830). This rocky intertidal site also includes abundant predatory gastropods, octopods, and crabs.

Host Preferences and Feeding Traces: Twenty-three individuals of *Vitularia salebrosa*, with shell lengths ranging from 40.5 to 54.1 mm, were observed under boulders at Venado Island in August 2005. Fourteen of these, all females, were found to be actively feeding on molluscan prey, which was determined by observing whether the proboscis could be seen extending through a hole in the host's shell as the predator was lifted away. All fourteen *V. salebrosa* and their hosts were collected and preserved in 75% ethanol. Five host shells (three oysters and two vermetids) were cut with a rock saw to view predation traces in cross-section. All figured material is housed in the Paleontological Research Institution (PRI) in Ithaca, NY. Non-figured material associated with experiments in this study (see below) is in the collection of the third author (HF). All other field-sampled material discussed herein is reposited in the collections of the first two authors (GSH and GPD).

Duration of Interactions with Molluscan Hosts: We estimated the duration of interactions between *V. salebrosa* and its hosts using two independent methods. The first, stable isotope sclerochronology, provides an indirect estimate but measures interactions with hosts under natural conditions in the field. The second approach, a long-term feeding experiment in the laboratory, cannot fully simulate natural conditions in the field but provides the only practical means of obtaining direct observations for attacks lasting months or longer. The two approaches together are much stronger than either alone. In this study, they yielded similar results on the estimated duration of species interaction.

Stable Isotope Sclerochronology: Stable isotope sclerochronology is a powerful tool for aging molluscan shells. The ratio of ^{18}O to ^{16}O isotopes in individual growth increments of shell $CaCO_3$ is determined by the environmental conditions in which shell precipitation occurs. In general, more positive/negative $\delta^{18}O_{carbonate}$ values correspond to cooler/warmer temperatures. The specific relationship between temperature and $\delta^{18}O_{carbonate}$ values has been empirically derived, with a change in isotope values of 1‰ being roughly equivalent to a temperature change of 4°C (Epstein et al., 1951; Krantz et al., 1987; Wefer and Berger, 1991; Jones, 1998). Salinity may also influence $\delta^{18}O_{carbonate}$ values via riverine input to coastal areas during the rainy season, which introduces freshwater that is relatively depleted in ^{18}O (Epstein et al., 1951; Surge et al., 2001, 2003).

When a shell is sampled serially across any axis of accretionary growth (e.g., umbo to ventral margin or across laminae of a thickened shell lip, etc.), the $\delta^{18}O$ values of those samples plotted against growth distance should exhibit near-sinusoidal variation resulting from seasonal changes in temperature and salinity over a year (Grossman and Ku, 1986; Wefer and Berger, 1991; Kirby et al., 1998). In the tropical eastern Pacific, where the rainy season coincides with warm summer temperatures, temperature and salinity effects on $\delta^{18}O_{carbonate}$ values reinforce one another and exaggerate the amplitude and distinctiveness of annual cycles in the profile (Geary et al., 1992). Annual cycles in oxygen isotope profiles can be counted to reconstruct a minimum estimate of lifespan and an absolute duration of shell growth. Here, we use the technique to age only new shell growth in bivalve hosts spanning the time between the initiation of an attack by *V. salebrosa* and the time the attack was disrupted when we collected the interacting species pair in the field.

Of the host-types available for this study, the stable isotope technique works best for determining duration of interactions with the oyster *Ostrea* cf. *fisheri*. *Vitularia salebrosa*'s edge drilling attacks on this oyster fortuitously mark the surface of the thickened lip. The $\delta^{18}O$ values of shell deposited between this point and subsequently formed growth increments at the lip record the duration of the attack. If attacks by *V. salebrosa* last roughly a week, as is typical of most muricid predators, there should be few or no growth increments formed by the host after the edge attack is initiated. Furthermore, the $\delta^{18}O$ profile of samples collected across any growth increments that did form should show little or no variation, consistent with the rate of environmental change

expeeted over a week. In contrast, if the duration of interaetions are on the scale of months or longer, there should be numerous growth increments formed after the attack is initiated, and the $\delta^{18}O$ profile should exhibit a roughly sinusoidal trend with a range of values expeeted of seasonal to annual variation. The two oysters seleeted for analysis were collected *during* an attaek in progress by *V. salebrosa* in August 2005. This eliminated any ambiguity over the provenanee of the feeding traces. However, beeause the attaek was interrupted, isotope profiles of these shells yield only a minimum estimate of the duration of predatory interactions by *V. salebrosa*.

The predicted annual range of $\delta^{18}O_{aragonite}$ for shells precipitated in nearsurface waters (top 20 m) of the Gulf of Panama is roughly –0.5 to –3.0‰, with an amplitude of 2.5‰ (Geary et al., 1992). Because oyster shell laminae are composed of calcite, a mineral form that differs in its isotopic composition from aragonite by a –1.0‰ offset (Bohm et al., 2000), the predieted annual range of oyster $\delta^{18}O_{calcite}$ for near surfaee waters of the Gulf of Panama is eloser to –1.5 to –4.0‰. Measured $\delta^{18}O$ values from a gastropod *Strombus gracilior* eolleeted at a tidally exposed beach near Venado Island have a larger amplitude of 4.5‰ for the strombid's first year of growth (Geary et al., 1992). For intertidal oysters at Venado Island (a slightly deeper site than the tidally exposed beaeh), the amplitude of annual ehange in the $\delta^{18}O$ profile should fall between 2.5 and 4.5‰, but probably closer to the latter.

Prior to sampling, oyster shells were soaked in a concentrated solution of bleach for 30 minutes, scrubbed with a soft brush, and sonieated in deionized water to remove organic contaminants, sediment, and encrusting organisms. Powdered carbonate samples were eollected by abrading the edges of individual laminae exposed at the outer lip with a modified 0.5 mm bit attaehed to a hand-held Dremel drill. Samples were also taken from laminae visible along the less exposed inner surfaee of the lower valve 1–2 mm from the edge of the outer lip. Powdered carbonate samples ranged from 50 to 80 µg in size, with an average spatial resolution of 0.5 mm.

Stable isotope measurements were made on a ThermoFinnigan Delta+XL IRMS in dual-inlet mode coupled to a Kiel-III carbonate preparation system housed at the University of South Florida College of Marine Seience. All values are reported in standard delta (δ) notation relative to the VPDB isotopic standard, where $\delta = [R_{sample}/R_{standard} - 1] \times 1000$ and R_{sample} and $R_{standard}$ are the oxygen isotopic ratios of the sample and the Pee Dee Belemnite (V-PDB) standard, respectively, in ‰ units. Stable isotopic precision, based on daily measurements of laboratory standards ($N > 500$) over the past 12 months, is ±0.06 ‰ (1 sigma) for oxygen, ±0.03 ‰ (1 sigma) for carbon.

Long-term Laboratory Observation of Feeding: An informal feeding experiment was eonducted at the Smithsonian Tropical Research Institute (STRI) marine lab at Naos, Panama by one of us (HF) to determine whether attacks last longer than one to two weeks. Three 37.9 liter aquaria with flow-through seawater dripped in from above were partitioned into equal quadrants with plastic netting. Three quadrants of each aquarium were used to house *V. salebrosa* and potential mollusean hosts, and the fourth quadrant contained a pipe for outgoing water. Each quadrant held one *V. salebrosa* and one host.

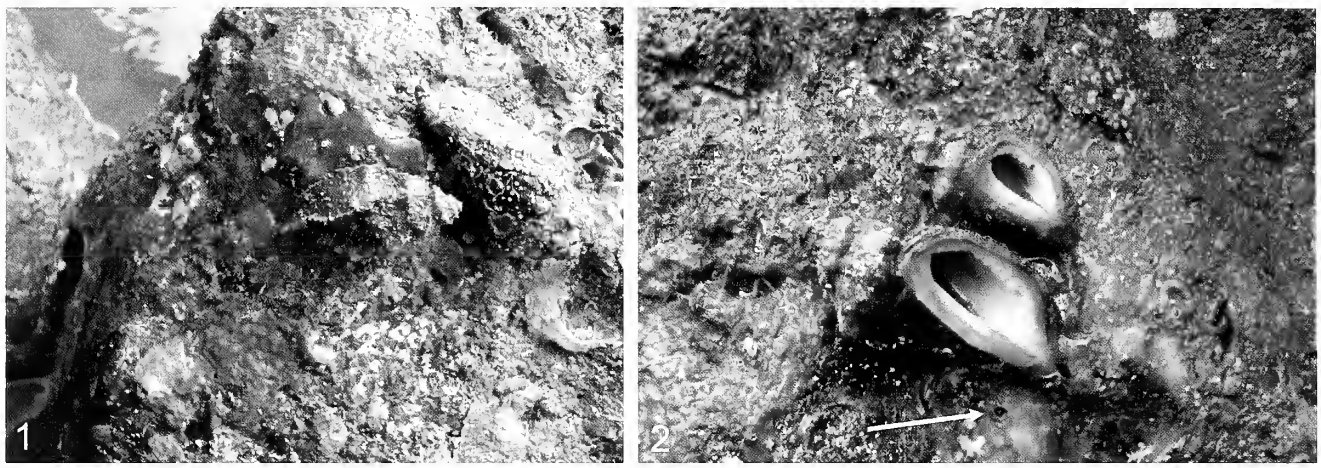
Each aquarium was a replicate of the other two in terms of the host type offered in each quadrant. *Vitularia salebrosa* in quadrant I of each aquarium were offered only the byssate oyster *Pinetada mazatlanica;* the vermetid *Tripsyeha (Eualetes) tulipa* was the sole host type offered in quadrant II; and either of the cementing oysters *Spondylus calcifer* or *Chama* sp. were offered in quadrant III, depending on availability. All four species are commonly found in the natural habitat of *V. salebrosa*. The experiment began April 18, 2006 and was terminated September 4, 2006. Observations were made roughly biweekly during this period. Hosts killed were replaced immediately with a single individual of the same speeies. Three *V. salebrosa* that died during the experiment were also replaced, but none died during attacks in progress. Twelve *V. salebrosa* were used in all.

RESULTS

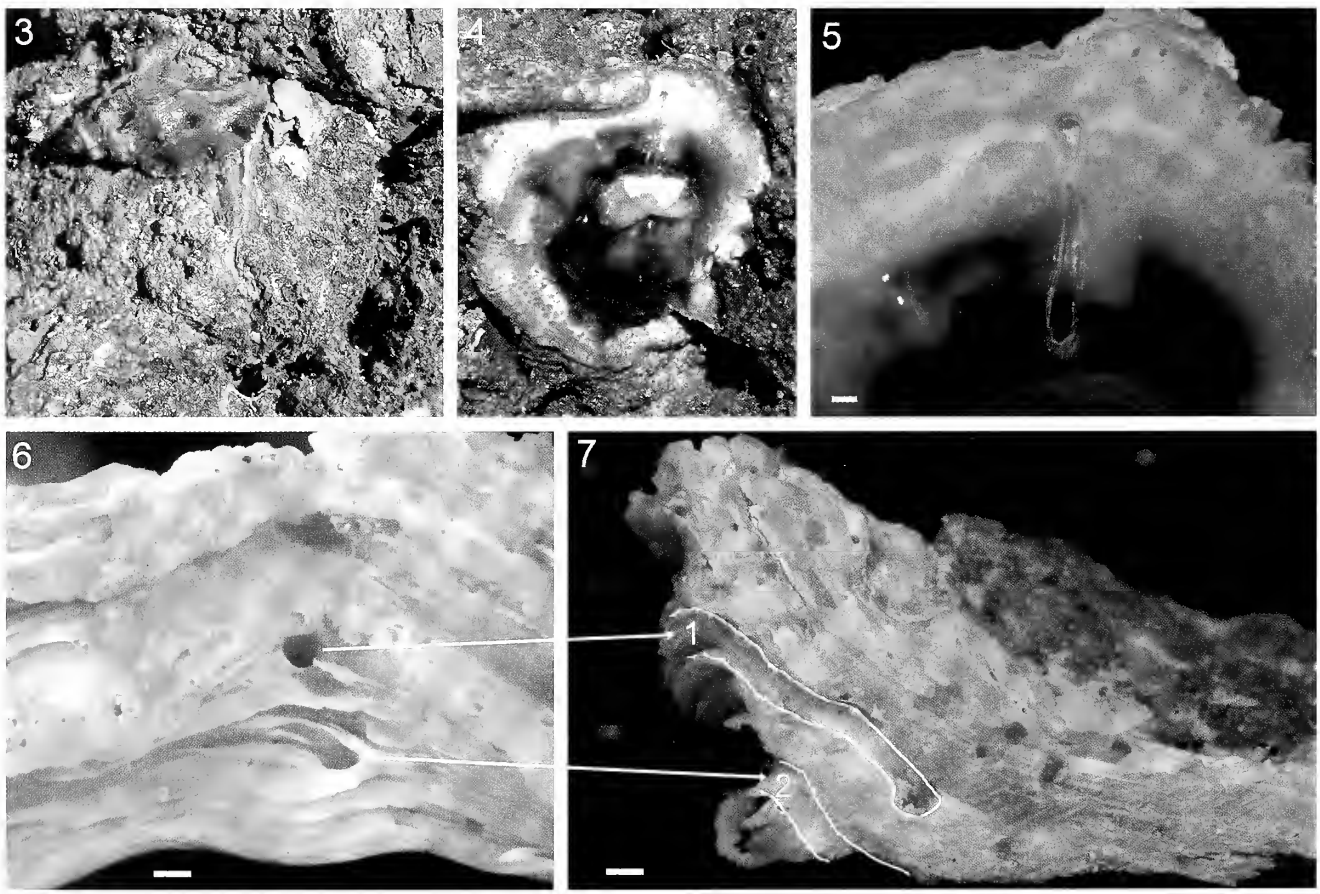
Ectoparasitism Feeding Traces: We observed fourteen *V. salebrosa* feeding on the following molluscan hosts during two low tides at Venado Island in August 2005: the oyster *Ostrea* cf. *fisheri* (n = 8), the calyptraeid gastropod *Crueibulum (Crueibulum) spinosum* (n = 2), and the vermetid gastropod *Tripsycha (Eualetes) tulipa* (n = 4). In nine instances, a large female *V. salebrosa* was joined by a single smaller male, which sat directly adjacent to the female (Figures 1–2). We observed only females feeding. Adjacent males were not situated over separate feeding holes.

Oyster Hosts (Figures 3–11): The following are general eharacteristics of interaetion traces associated with the eight *Ostrea* collected from the field: *Vitularia salebrosa* was situated on the left, cemented valve, near the ventral eommissure, with its proboseis extended through a straight-sided, 1 mm diameter hole that penetrated into the lip of the left, cemented valve at an angle parallel to the commissural plane. The lower half of the hole (the half closest to the rock substrate) euts through multiple oyster lamellae, as if formed by a drilling attack, while the upper half (the half closest to the commissural plane of the oyster) does not. Instead, the roof of the hole is formed by a single curved lamina, apparently as the oyster deposited new shell over the feeding proboscis. The attaek, therefore, must have initiated as an edge drilling attack at an older (ontogenetically earlier) commissure.

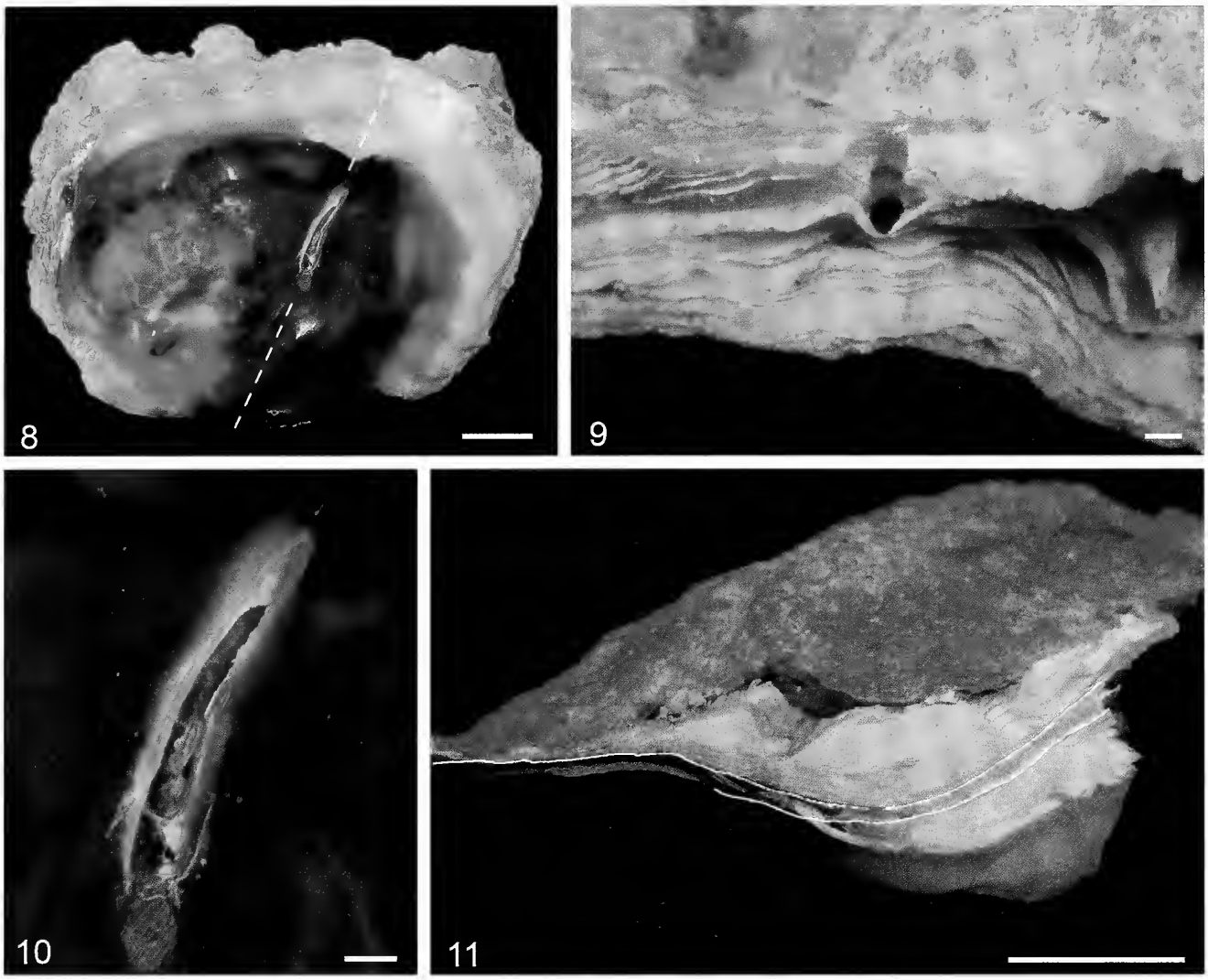
The hole through which *Vitularia salebrosa* feeds travels into the lower valve as a tunnel, curving gradually until it erupts at the inner surface some distance from the lip. From there, the tunnel continues in a straight



Figures 1–2. Large female *Vitularia salebrosa* with smaller male on overturned boulder. Female was feeding on an oyster heavily encrusted with bryozoans and sponges. Figure 2 shows hole (arrow) leading to feeding tunnel and characteristic foot scar left by female (etched area around hole). Adjacent male was not feeding.



Figures 3–7. Ectoparasitism traces left by *Vitularia salebrosa* feeding on the oyster *Ostrea* cf. *fisheri* (PRI 8743). **3.** Female ectoparasite feeding on oyster attached to intertidal boulder. **4.** Left valve of oyster host showing opening of feeding tunnel (hole near screwdriver tip) and feeding tube extending from hole to adductor muscle. **5.** Close-up of hole and feeding tube. **6.** Close-up of left valve showing calcite foot scar (top, left of center) and holes leading to two feeding tunnels. Valve is oriented with commissure at bottom of image. **7.** Cross-section of oyster shell revealing two feeding tunnels. Only the second tunnel provided access to the interior of the hosts shell at the time of collection. Scale bars = 1 mm.



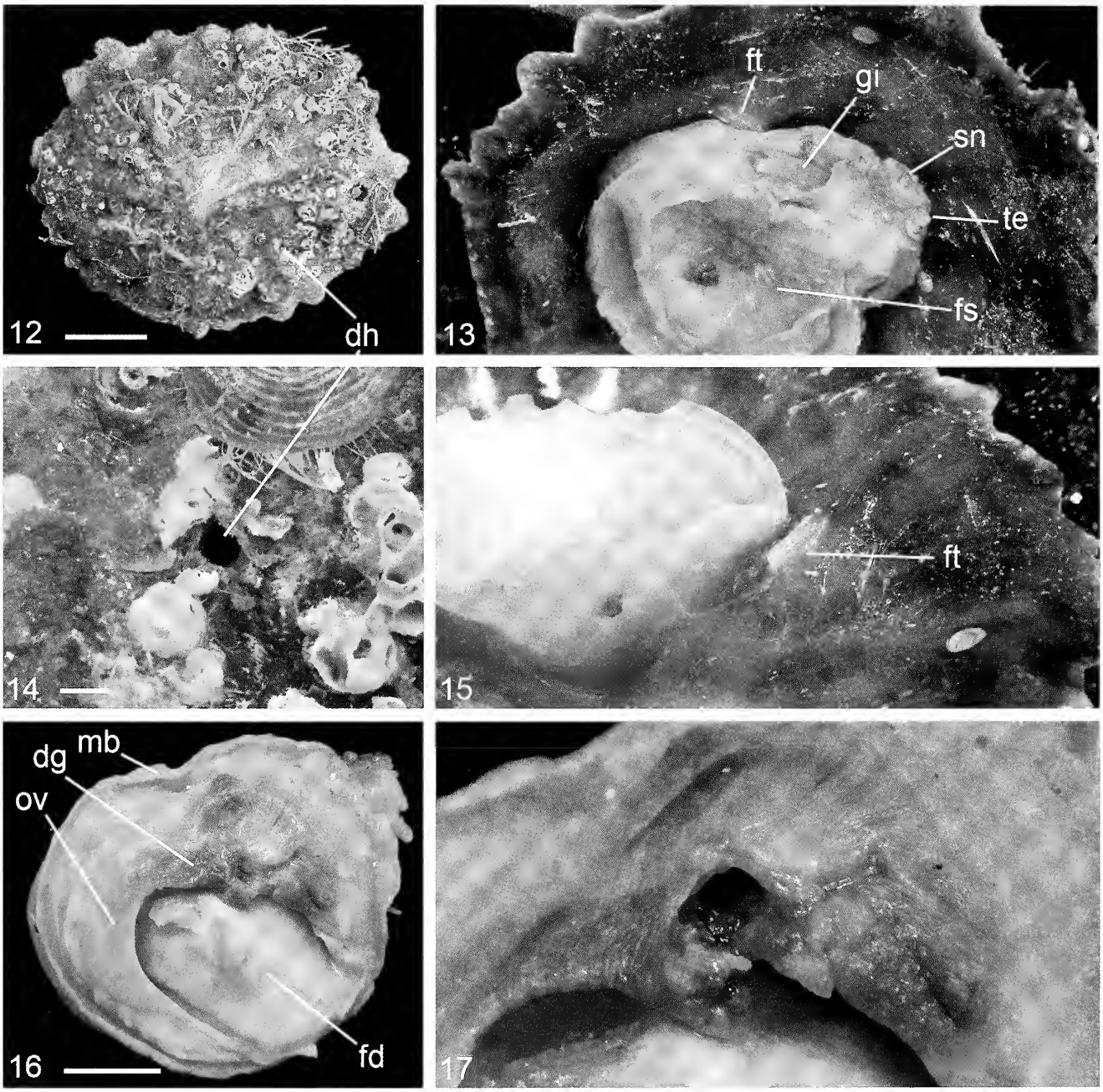
Figures 8–11. Ectoparasitism traces left by *Vitularia salebrosa* feeding on the oyster *Ostrea* cf. *fisheri* (PRI 8744). **8.** Left valve of oyster prey showing feeding tube leading towards adductor musele. Dotted line depiets cut made for cross-section in figure 11. **9.** Close-up of external hole showing upper lip of hole excavated by drilling and lower lip formed by undulating shell laminae deposited by oyster. **10.** Close-up of feeding tube on interior of oyster. **11.** Cross seetion of oyster shell revealing a single, long feeding tunnel winding through shell. Outer lip of oyster is to the right of the image. Scale bars in figures 8 and 11 = 5 mm. Scale bars in figures 9 and 10 = 1 mm.

line as a closed tube or open channel with low walls. The tube/channel structure extends up to 25 mm along the inner surface stopping just inside the margin of the adductor musele sear. There was no sign of feeding on the adductor musele itself, although some muscles exhibited a loealized whitened region that could represent scar tissue or inflammation.

A cross-section of the oyster in Figure 3 shows two separate tunnels, although just one penetrated the inner surface of the valve. The termination point of the earlier tunnel (tunnel #1 in Figure 7) occurs at preeisely the same growth line that the newer tunnel (tunnel #2 in Figure 7) begins. Feeding activity by *V. salebrosa* on this host is interpreted to have been more or less continuous, with the seeond tunnel beginning almost immediately after abandonment of the first tunnel. Adjacent to the outer hole leading to tunnel #1 is a cap of bubbly ealcite eement, whieh was formed underneath the foot of the predator (a foot scar). No other oyster valves were found with a foot scar.

Calyptraeid Gastropod Hosts (Figures 12–17): Two *Crucibulum (Crucibulum) spinosum* were found with a single *V. salebrosa* sitting on top of the host shell with its proboscis extending through a 1 mm diameter, straight-sided hole roughly 7.5 mm from the shell lip. No foot scars on the outer surface of the host shells were observed. The hole, which is perpendicular to the shell surface, erupts ventrally as a tube that runs along the inner surface of the shell, adjacent to the cup, for about 5 mm. The distal, open end of the tube exits between

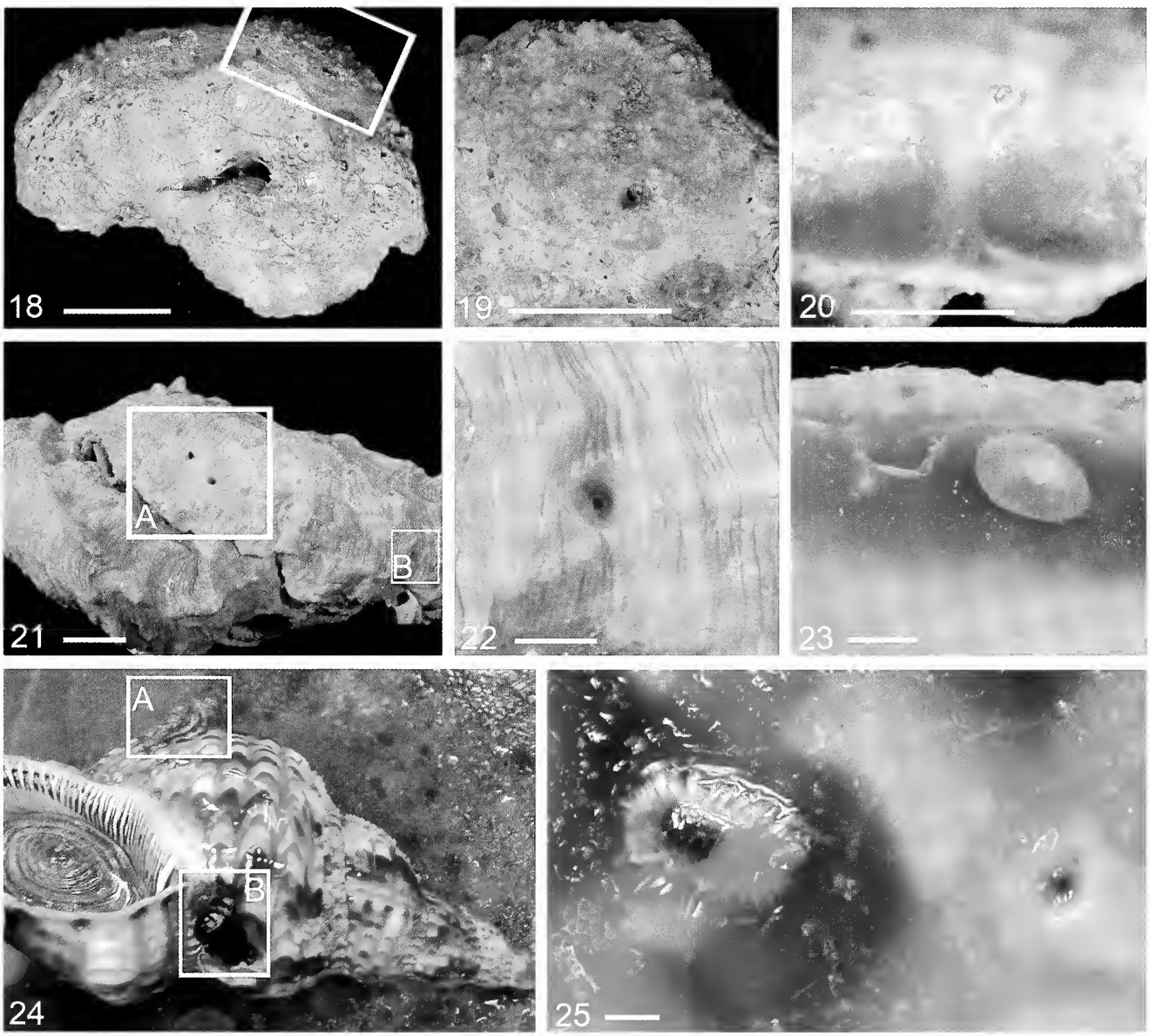
the shell and mantle in the region just posterior to the host's head and gills but continues as a low-sided channel extending another 5 mm. Dissection of both individuals revealed a cavity in the digestive gland roughly 2 mm in diameter and 5 mm in length, apparently representing the region of the gland consumed by *V. salebrosa*. The cavity did not break through the digestive gland but terminated within it. A second drill hole that was repaired and is not associated with a tube is present on one of the shells, although the driller responsible for this hole is not known. No foot scars were found on any calyptraeid shells.



Figures 12–17. Ectoparasitism traces left by *Vitularia salebrosa* feeding on the calyptraeid gastropod *Crucibulum* (*Crucibulum*) *spinosum* (PRI 8745). **12.** Dorsal view of *Crucibulum* shell. 13. Ventral view of *Crucibulum* showing position of feeding tube relative to animal. **14.** Close-up of external opening of drill-hole. **15.** Close-up of feeding tube with animal removed; feeding tube (not including etched area beyond tube) is roughly 5 mm in length. **16.** Dorsal view of *Crucibulum* anatomy showing damaged digestive glands. **17.** Close-up showing hollowed-out digestive glands. Abbreviations: **dg,** digestive glands; **dh,** drillhole; **fd,** foot, dorsal side; **ft,** feeding tube; **fs,** foot, sole; **gi,** gills; **mb,** mantle border; **ov,** ovaries; **sn,** snout; **te,** tentacle. Scale bar in figure 12 = 10 mm; scale bar in figure 14 = 1 mm; scale bar in figure 16 = 5 mm.

Vermetid Gastropod Hosts (Figures 18–23): All four *Tripsycha (Eualetes) tulipa* hosts were attacked by drilling through the shell wall. Drillholes are roughly 1 mm in diameter and conical in vertical cross-section. Figures 18 (box) and 19 show an attachment scar from the foot consisting of a broad halo of heavy shell dissolution capped by a smaller region of reprecipitated calcite cement. Sectioning of this shell revealed that the hole on the outer surface was connected to a tube on the inner surface (Figure 20). Vermetids were observed with as many as seven complete and incomplete holes. Figure 21 shows a shell that had seven holes, although only three are visible from a single angle (two in box A, and one in box B); all but one of the holes are incomplete or repaired (Figures 22–23).

Other Molluscan Hosts (Figures 24–25): In a holding tank used for teaching at the STRI marine lab at

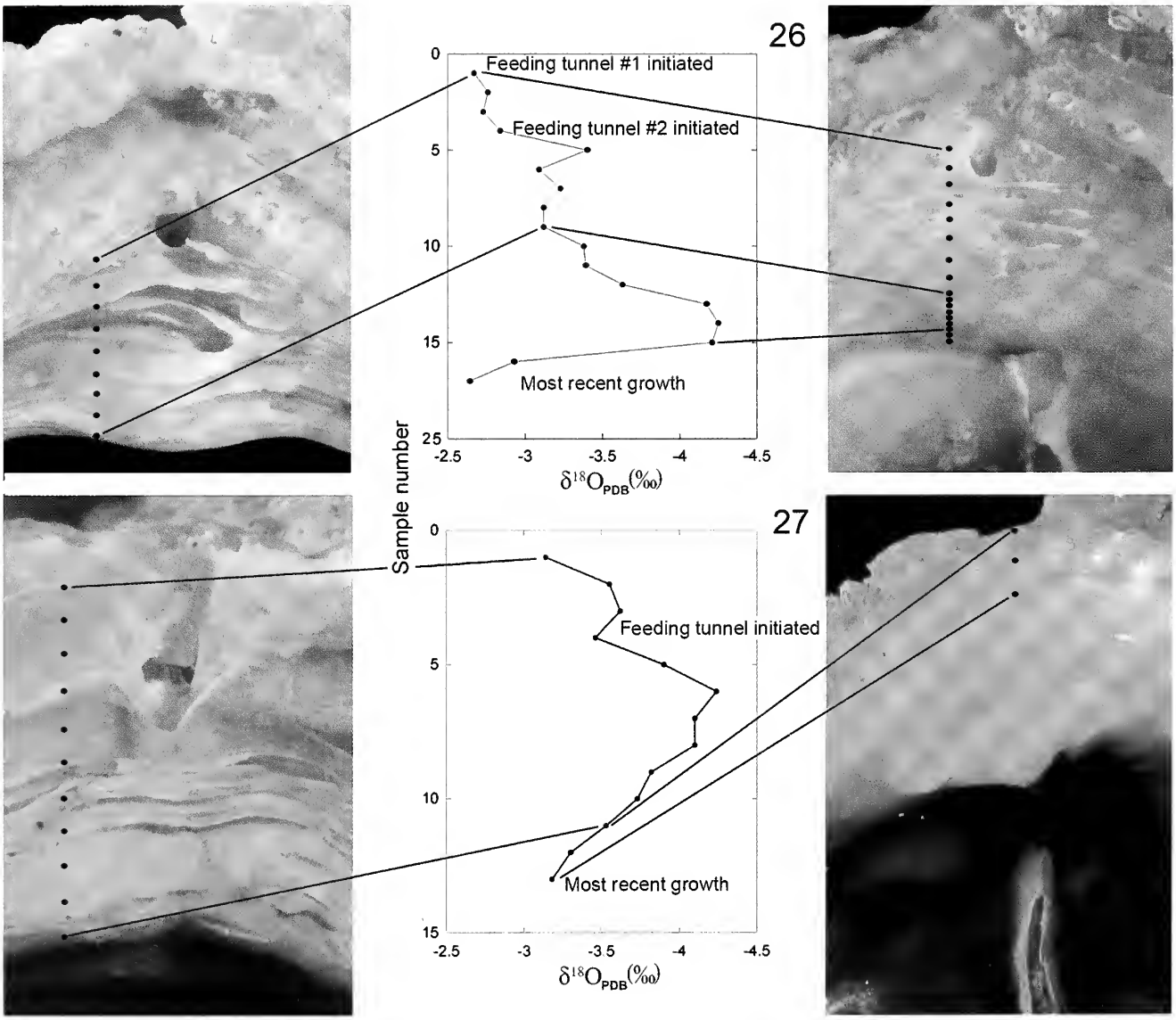


Figures 18–25. Ectoparasitism traces left by *Vitularia salebrosa* on the vermetid gastropod *Tripsycha (Eualetes) tulipa* (PRI 8746: figures 18–20; PRI 8747: figures 21–23) and the ranellid gastropod *Charonia tritonis*. **18.** Top-down view of vermetid shell; foot scar highlighted in box. **19.** Close-up of foot scar and drillhole. **20.** Close-up of feeding tunnel on interior surface of sectioned vermetid shell. **21.** Second vermetid shell showing three drillholes (2 in box A, 1 in box B). **22.** Close-up of drillhole in box B. **23.** Close-up of interior surface of sectioned vermetid shell showing internal shell repair of two holes corresponding with drillholes in figure 21, box A. **24.** Large *Charonia tritonis* gastropod attacked by two *V. salebrosa* predators in a holding tank at STRI marine lab, Naos, Panama. **25.** Close-up of two drill holes. Ectoparasite in figure 24, box A observed feeding through smaller, rounded hole on the right side of the figure (see text for details). Scale bar in figure 18 = 10 mm; scale bars in figures 19–21 = 5 mm; scale bars in figures 22–23 and 25 = 1 mm.

Naos, Panama, a large *Charonia tritonis* gastropod was attaeked by two *V. salebrosa*. One individual was removed to reveal two adjacent drillholes and its long proboscis extending through one of them. The hole is round in plan view and eonieal in vertieal cross-section. The second hole is irregular, having a round inner edge but a strongly ovate outer edge. Additionally, the wall of the second hole is heavily gouged with what appear to be radular scrape marks. We do not know when these attacks started or if they resumed at a later date. *Charonia tritonis* generally occurs in slightly deeper waters than *V. salebrosa* and is almost certainly a novel host.

Stable Isotope Sclerochronology: The first oyster analyzed (PRI 8743: same shell as in Figures 3–7) was sampled along the axis of lip thiekening, with sample 1 eorresponding roughly to the point at whieh feeding tunnel #1 was initiated by drilling, sample 4 corresponding to the initiation of tunnel #2, and sample 17 (the last sample) corresponding to the most recently deposited shell lamina, closest in time to when the attaek was interrupted by our colleetion of both ectoparasite and host (Figure 26). Nearly eonstant isotope values between samples 1–4 suggest that abandonment of tunnel #1 and initiation of tunnel #2 oeeurred over a very short period of time and, thus, without any signifieant break in feeding activity.

The isotope profile of this first set of samples shows a single, complete cycle, with values beginning at –2.6‰ (sample 1) followed by a warming/freshening trend with



Figures 26–27. Oxygen stable isotope sclerochronology profiles of two oyster shells (*Ostrea* cf. *fisheri*) parasitized by *Vitularia salebrosa*; Images to the right and left of each profile show approximate spacing and position of samples taken from each oyster. Figure 26 shows same oyster as in figures 3–7 (PRI 8743). Figure 27 shows same oyster as in figures 8–11 (PRI 8744). See text for details.

a minimum value of –4.3‰ (sample 14) and a return to cooler/drier values around –2.6‰ (sample 17). This pattern, together with the 1.7‰ magnitude of variation between isotopic maxima and minima, is consistent with seasonal change, probably on the scale of months. To refine this estimate of the duration of the attack, we divided the observed amplitude of the oyster profile (1.7‰) by the predicted annual amplitude of 2.5‰ for near-surface waters of the Gulf of Panama (see Materials and Methods). By this comparison, the observed data encompass roughly 65% of the expected annual range, or 7.8 months.

A more conservative estimate of the attack duration can be obtained by dividing the amplitude of the oyster profile (1.7‰) by the larger annual amplitude (4.5‰) reported for the isotope profile of a strombid gastropod collected at Venado Beach, a more exposed site than Venado Island that has greater environmental extremes of temperature and salinity (Geary et al., 1992) (see Materials and Methods). By this estimate, the amplitude of isotopic variability in the oyster profile is roughly 38% of the observed annual amplitude in the strombid profile, or 4.6 months.

The isotope profile of the second oyster (PRI 8744: same shell as in Figures 8–11) shows a similar full-cycle but a lower amplitude (1.0‰) due to truncation of isotopically heavier values at the beginning and end of the profile (Figure 27). The amplitude of this second oyster profile is roughly 40% of the annual range predicted for near surface waters of the Gulf of Panama, or 5.0 months. If this profile is compared to the observed annual range of 4.5‰ for the Venado Beach site, the estimate for the duration of the attack is a more conservative 2.6 months.

Long-term Feeding Experiment: We observed a total of 8 long-term interactions between *V. salebrosa* and its hosts (0 attacks on *Pinctada mazatlanica*, 1 attack on *Spondylus calcifer*, 3 attacks on the vermetid gastropod *Tripsycha (Eualetes) tulipa*, and 4 attacks on *Chama* sp; Table 1). The average duration of attacks that were completed (i.e., ending in the death of the host) was 46 days (n = 7). The shortest attack recorded was on *Chama*, lasting 21 days. The longest attack, also on *Chama*, lasted 103 days and was still in progress at the termination of the experiment. The longest attack on a vermetid lasted 69 days. The only attack on *Spondylus* lasted 44 days. *Vitularia salebrosa* were observed to move on and off of their host in half of the observed encounters. In one case, an attack on *Tripsycha* was abandoned for a month before resuming at the same position. In a 97-day attack on *Chama*, two *V. salebrosa* sat side by side on a single host and fed from a single hole (one snail had climbed over the experimental partition in the tank).

DISCUSSION

In this study, we show that a typical interaction between *Vitularia salebrosa* and its mollusean hosts is initiated by wall- or edge-drilling and lasts several months. Estimates from isotope sclerochronology of two *Ostrea* hosts collected in the field during attacks in progress indicate that the interactions had already lasted between a minimum of two and a maximum of eight months when we collected the species pairs. Had the attacks not been interrupted, they might have lasted considerably longer. In our laboratory-based feeding experiments, we observed an attack lasting 103 days, which ceased only because the experiment was terminated at this time. Both estimates of feeding times for *V. salebrosa* exceed a 29-day long attack recorded for the drilling muricid *Trophon* in the Antarctic (Harper and Peck, 2002) and are on par with the nearly half-year long attacks recorded in the laboratory for the muricid *Genkaimurex varicosa* (Kuroda, 1953), which has been regarded as ectoparasitic or commensal on scallops in deep waters off Japan (Matsukuma, 1977). Although we did observe some mortality of hosts due to attacks by *V. salebrosa* in our laboratory experiment, death was in all cases delayed well beyond the initiation of feeding. Table 1 shows that *Spondylus* and *Tripsycha* hosts survived, on average, for 44 days after feeding began, while *Chama* survived for an average of 63 days. By contrast a typical predatory muricid consumes its entire prey within hours after feeding begins, and drilling attacks rarely last longer than a week (Dietl and Herbert, 2005; Herbert, unpublished experimental observations). Combined with field data and isotope results, these observations suggest that *V. salebrosa* is best characterized as an ectoparasite than as a predator.

Ectoparasite as used here refers to an organism that lives on the exterior of and takes resources from another organism in a lasting, intimate interaction that may or may not be lethal. Ectoparasites that have the capacity to move between hosts minimize the fitness losses associated with

Table 1. Results of Long-Term Feeding Experiment

Species	Number of Attacks	Mean Duration of Attacks (days)	Minimum Duration of Attacks (days)	Maximum Duration of Attacks (days)
Pinctada mazatlanica	0	-	-	-
Spondylus calcifer	1	44	44	44
Tripsycha tulipa	3	44	31	69
Chama sp.	4	63	21	103*

*experiment terminated before death of prey/host

intense use of host resources and host death (Lehmann, 1993; Ewald, 1995). This generalization may help explain why the majority of hosts offered in our experiments were ultimately over-exploited (killed) by *V. salebrosa*.

In the following sections, we discuss the interaction traces, specialized anatomy, and reproductive behavior of *V. salebrosa* relative to other predatory Muricidae that are also suggestive of an ectoparasitic lifestyle.

Interaction Traces of an Ectoparasitic Muricid: Foot Scars, Feeding Tunnels, and Feeding Tubes: The foot of *Vitularia salebrosa* frequently forms an attachment scar on the host shell that consists of a circular calcareous deposit (or "carbonate foot pad" of Bromley and Heinberg, 2006) or a region of substrate etching. Such scars are exclusive to gastropods that have a sedentary existence on molluscan hosts or rock substrates (e.g., herbivorous limpets: Bromley and Heinberg, 2006; capulid gastropods: Matsukuma, 1978; Ward and Blackwelder, 1975; Bongrain, 1995; suspension feeding calyptracid gastropods: Walker, 1992; Simone, 2002; Santos et al., 2003; detritivorous hipponicid gastropods: Noda, 1991; Vermeij, 1998; Simone, 2002; Santos et al., 2003; and ectoparasitic muricids, including *Genkaimurex* and some coralliophilines: Matsukuma, 1977; Massin, 1987). The mechanism of attachment likely explains the formation of the scars. In general, scar formation is a function of organic adhesives secreted by the gastropod foot that contain a high concentration of proteins with acidic or basic residues (Smith et al., 1999; Smith, 2001; Pawlicki et al., 2004; Bromley and Heinberg, 2006). The low or high pH of these residues produces etching or secondary calcite deposition, respectively. The formation of foot scars by *V. salebrosa* suggests that it, like *Genkaimurex*, has evolved the capacity to secure itself to host shells and has a sedentary life habit, both of which are highly unusual for the Muricidae.

Other telltale signatures of prolonged feeding by *V. salebrosa* are the calcareous tunnels and tubes through which its long proboscis extends during feeding. One of the first questions we attempted to address was whether tunnels and tubes are formed during feeding by *V. salebrosa*, or whether this ectoparasite simply takes advantage of pre-existing openings in prey shells left by other organisms. It is well known, for example, that calcified infestation tunnels roughly the same diameter as those used by *V. salebrosa* are bored into oysters by spionid polychaetes (Huntley, 2007). Spionid tunnels, however, are u-shaped borings, where the worm penetrates into the shell lip and then turns 180 degrees, emerging at the lip adjacent to the initial boring (Blake and Evans, 1973). These and other organic-walled spionid structures (e.g., Ishikawa and Kase, 2007) are, thus, easily distinguished from the calcareous feeding tunnels and tubes of *V. salebrosa*, which proceed in a direct line from the lip to the targeted tissues or organs. All indications are that the structures used by *V. salebrosa* are formed *during* the interaction between this ectoparasite and its host.

A second question was whether feeding tubes used by *V. salebrosa* and which extrude on the internal surface of some prey shells are made by *V. salebrosa* or its hosts. At least two muricids do, in fact, secrete protective calcareous tubes around their proboscises. In both cases, the muricids [*Reliquiaecava robillardi* (Liénard, 1870) and *Magilus antiquus* Montfort, 1810] are coralliophilines parasitic on corals, and the proboscis is embedded within the host tissues (Massin, 1987; M. Oliverio, personal communication to GSH, 28 Jan. 2008). Feeding tubes associated with *V. salebrosa*, however, are formed by a shell layer that is continuous with the inner surface of the host's shell and presumably formed *by the host* in a process analogous to pearl formation in oysters. The host simply deposits a thin layer of shell over the intruding proboscis in an attempt to seal off the irritant, which results in a straight, calcareous-walled tube.

From time to time, shell repair by the host is effective, with feeding tunnels and drillholes being completely sealed off. In our laboratory feeding experiments, *V. salebrosa* would often leave its host for short intervals, and it may be that successful repair is possible during these breaks in activity. This would force *V. salebrosa* to abandon its host, punch through the repair, or drill a new hole. Some hosts, especially vermetids, have been found still alive with multiple repaired holes. We found one vermetid in the field with six repaired holes and one unrepaired hole (still being used by *Vitularia salebrosa*). Also, at least in edge-drilled oyster hosts, layer after layer of shell may be deposited over the intruding proboscis, such that the original edge-drilled hole is displaced 5 mm from the new commissure.

Persistent ectoparasites, however, are clearly able to maintain open feeding tunnels even after intense efforts by the host at internal shell repair. Tubes that are kept open even with thick shell layers deposited over most of the length of the proboscis by the host become tunnels *through* the prey shell. How tunnels are kept open is unknown. An unusually long accessory boring organ (ABO) peduncle could be used to maintain internal openings in some tubes. Our initial study of *V. salebrosa*'s anatomy found that it does indeed possess a relatively narrow and long ABO (Simone et al., 2009). However, this solution is unlikely to work for some of the longer tunnels, which can reach nearly 25 mm in length. It is also problematic for radular rasping alone to maintain the opening. Carriker and Van Zandt (1972) found that muricid drillers that have had their ABO's amputated cannot excavate deep holes in shells until the ABO has regenerated. Herbert et al. (2008), however, showed that *V. salebrosa* sometimes forms a robust, elephant-tusk shaped radula that is different from its typical radular morphology and unique within the Muricidae. It is possible that this unusual morphology could function more effectively as a drilling implement in the absence of ABO secretions, particularly when host scars are newly formed and thin or largely proteinaceous in composition.

A third possible mechanism for preventing host shell repair of deep feeding tunnels is that *V. salebrosa*

produces shell dissolving/loosening secretions from the proboscis itself. This occurs in cassid drillers, for example, which have two large salivary glands that open into the proboscis and trickle acids to the site of boring on echinoid prey (Carriker and Gruber, 1999). *Vitularia salebrosa* has several glands that could potentially function in this manner, including the salivary glands, the gland of Leiblein, the glandular part of the valve of Leiblein, and the gland of the posterior esophagus (Simone et al., 2009). A precedent for specialized boring glands of the proboscis already exists in coralliophiline and some rapanine muricids, which can penetrate the epidermis of cnidarian prey with proteolytic enzymes secreted from a single salivary duct opening into the mouth (Ward, 1965; Fankboner, 1970). At least one coralliophiline muricid, *Reliquiaeeava robillardi*, reportedly uses secretions of the proboscis to bore holes through the aragonitic skeletons of coral hosts (Massin, 1987). Future histological work will be needed to test these ideas.

Anatomical Specializations for Ectoparasitic Feeding on Molluscan Hosts: Preliminary data on the anatomical specializations for an ectoparasitic mode of life suggest that *V. salebrosa* has a reduced buccal mass and radula, an elongate proboscis, and a highly simplified foregut relative to other members of the Muricidae. These aspects of the soft anatomy are documented and discussed in detail in a companion paper (Simone et al., 2009). All are consistent with specialized feeding on host fluids. In addition, Herbert et al. (2008) reported that few individuals of *V. salebrosa* (one in nine) collected in the field from museum collections actually possess a radula, an observation also made by D'Attilio (1991). All individuals we collected in August 2006, however, possessed a complete and functional radula (Simone et al., 2009). A similar situation occurs in *Genkaimurex varieosa*, with some studies reporting that this species possesses a radula (Matsukuma, 1977) and others reporting that it does not (Kuroda, 1953). It is possible that these ectoparasitic muricids only form a radula when necessary to initiate attacks by drilling, perhaps just once a year and perhaps seasonally. The radula could then be reabsorbed as the animal begins suctorial feeding. The only other muricids known definitively to lack a radula are ectoparasitic coralliophilines, which feed suctorially on cnidarians (D'Attilio, 1972). The muricine muricid *Pterymarchia martinetana* (Röding, 1798) may also lack a radula (D'Attilio and Myers, 1985), although nothing is known of this species' ecology.

However, prior reports that *V. salebrosa* lacks a radula are based on a potentially error-prone technique that involves not dissection but dissolution of head-region tissues of dried animals in concentrated potassium hydroxide. This technique is useful for extracting radulae from dried and poorly preserved museum specimens, but it is often impossible to determine whether such specimens are complete. Incomplete specimens are likely in the case of *V. salebrosa*, because the proboscis is long, extruding deep into the host shell, and might be severed during collection as the animal is pulled from the substrate.

Host Consumption by *Vitularia salebrosa* Targets Renewable Resources: In general, parasites must target renewable and energetically profitable food resources of a host in order to sustain a long-term interaction. *Genkaimurex*, for example, does not damage its scallop host's tissues and presumably feeds suctorially on replenishable "fluids" (Matsukuma, 1977), such as blood. Gastropods of the muricid genus *Vexilla* are ectoparasites on much larger echinoids and graze the epidermis, which may regenerate (Kay, 1979; Vaïtilingon et al., 2004). Coral ectoparasites of the muricid subfamily Coralliophilinae feed preferentially at the margins of coral colonies due to the tendency for renewable photosynthetic products to flow towards energy sinks at the colony margins (Oren et al., 1998). In short, wherever there is evidence of parasitic feeding by a muricid, there is evidence that the parasites target renewable resources of the host.

In this study, we found that *V. salebrosa* feeding tubes in oyster hosts stop just inside the outer margin of the adductor muscle scar, in the approximate location of a major blood vessel. We did not observe damage to oyster tissues, including the adductor muscle, and it is reasonable to conclude that *V. salebrosa* pierces these blood vessels and feeds suctorially. Direct feeding on the adductor muscle itself by *V. salebrosa* would be immediately lethal to the oyster, as the oyster would no longer be able to close its shell and defend itself from opportunistic predators. The consistency with which this anatomical region of the host was targeted (100% of oysters found with a *V. salebrosa* attached) is evidence that feeding on oyster hosts by *V. salebrosa* is highly specialized. *Vitularia salebrosa* derives nutrition from calyptraeid hosts differently, but some degree of specialization is evident here as well. The feeding tubes of both calyptraeids we dissected led in the direction of the digestive gland, and the organ itself had been partially hollowed out in each case. Digestive glands of Mollusks are commonly attacked by endoparasitic protists (Wardle, 1993; Damborenea et al., 2006; Gonzalez-Moreno and Gracenea, 2006), and some molluscan hosts can survive with infesting parasites occupying as much as 50% of the glands (Tetreault et al., 2000). Moore and Halton (1973) showed that molluscan hosts adapt to digestive gland infections with histochemical changes that increase intracellular digestive processes, which is the same response as in animals that are starved. Thus, digestive glands of calyptraeids constitute a potentially viable source of nutrition for a molluscan ectoparasite.

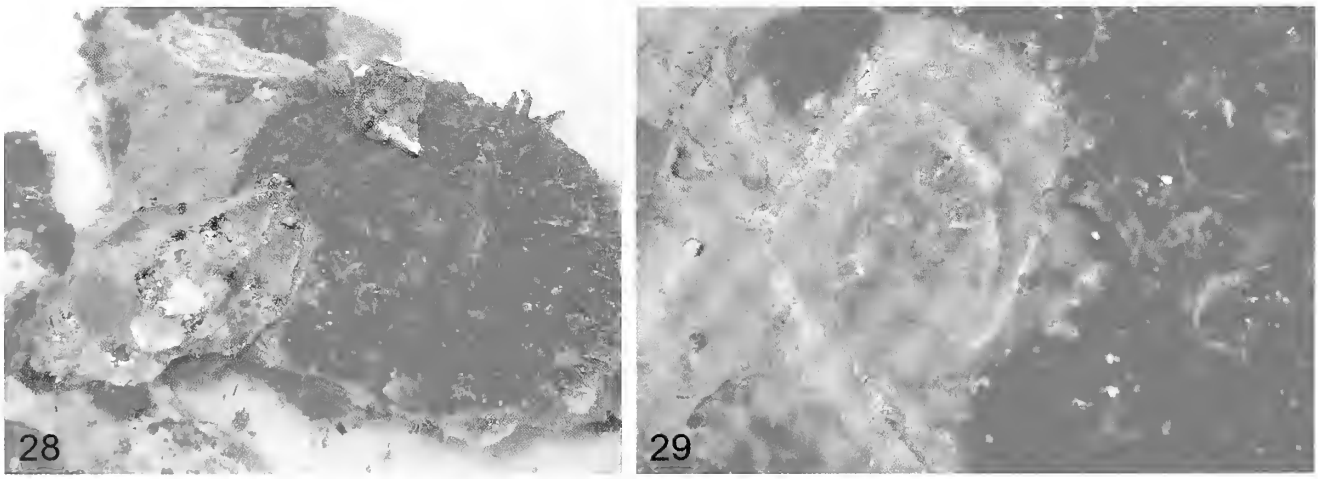
We have no data on organs, tissues, or fluids of vermetids that might be targeted by *V. salebrosa*. The fact that some vermetid hosts were drilled as many as seven times could mean that this interaction is less specialized than the others. However, unlike other hosts, vermetids can seal off damaged parts of the shell by calcareous septa. Doing so during an attack by *V. salebrosa* might

force the ectoparasite to drill a new hole. Also, formation of septa likely frustrates the drilling process of ectoparasites, which have little information on whether or not they are drilling into an empty chamber. The presence of occasional foot scars and feeding tunnels on vermetid hosts suggests that prolonged, non-lethal interactions with vermetids do occur in nature. In our feeding experiments, interactions between vermetids and *V. salebrosa* ranged from a few weeks to over two months. Shorter interactions may have to do with the relative sizes of ectoparasite and host, with smaller hosts less able to recover from feedings by large *V. salebrosa*. This hypothesis can be tested in the future in an experimental setting.

Reproductive Challenges for a Sedentary Ectoparasite: For an animal with internal fertilization, a parasitic and largely immobile existence poses a major problem for finding reproductive partners. Long-term commensals have evolved a variety of adaptations to deal with this challenge. The shrimp *Pontonia margarita*, a symbiont of the oyster *Pinctada mazatlanica* from the Pacific coast of Panama, for example, has evolved a system of social monogamy or mate guarding (Baeza, 2008). Calyptraeid and coralliophiline muricid snails, which are also sedentary, have evolved protandrous hermaphroditism, where new recruits become males in the presence of older females or females in the absence of any other females (Massin, 1990; Collin, 1995; Richter and Luque, 2004). In the case of *V. salebrosa*, many of the snails we observed in the field were in male-female pairs, which is consistent with both social monogamy and protandrous hermaphroditism. We observed a similar pairing behavior in the laboratory. Even though snails were housed individually in separate compartments, they would occasionally crawl out of the water and over barriers to form pairings with snails in neighboring compartments. When pairs did form in the lab, snails would sit side-by-side and occasionally swap positions over a single feeding hole. In the field, we observed only larger females over feeding holes. We also did not find any host shells with more than one foot scar or open feeding hole, suggesting that males are more mobile than females, and that when females and males are together, holes may be "shared."

Evidence for Ectoparasitism in the Indo-Pacific Congener *Vitularia miliaris*: Through personal communication to the senior author (GSH) in 2007, Anders Warén (Swedish Museum of Natural History) relayed that he has unpublished observations of identical ectoparasite feeding traces and adaptations in *Vitularia miliaris* (Gmelin, 1791), an Indo-Pacific species that feeds on bivalves, including *Isognomon* oysters in Australia and *Pinna* pen shells in the Philippines. Like *V. salebrosa*, *V. miliaris* interactions with bivalves result in the same diagnostic foot scar and feeding tunnel leading to the adductor muscle. Warén also remarked that *V. miliaris* exhibits protandrous hermaphroditism. Dr. Marco Oliverio ("La Sapienza" University Rome, Rome, Italy) has kindly provided photographs of *V. miliaris* collected from Vanuatu, reproduced here, that show a male-female pair and characteristic foot scar on a *Spondylus* host (Figures 28–29). Based on these observations, the origin of ectoparasitism in *Vitularia* dates back to at least the last common ancestor of *V. salebrosa* and *V. miliaris*. Evidence from the fossil record suggests that this ancestor predates the Late Miocene or Early Pliocene, or the approximate time when both species first appear in essentially modern form in the tropical western Atlantic (Vokes, 1977, 1986). It would not be surprising to find *Vitularia*-style interaction traces on oyster, vermetid, or calyptraeid hosts in the Late Oligocene of Europe, which is the approximate age of the earliest known species of *Vitularia* (Vokes, 1977).

Evolution of Ectoparasitism in the Muricidae: The evolution of ectoparasitism of molluscan hosts in the Muricidae is exceedingly rare, and the *Vitularia* case study provided in this paper is only the second example



Figures 28–29. *Vitularia miliaris* from Vanuatu, Indo-West Pacific, shown in male-female pair feeding on *Spondylus* spiny oyster. Figure 29 shows characteristic ectoparasite foot scar beneath the foot of the female.

ever documented. One reason for its rarity may have to do with the intensity of selection for faster feeding. Espeeially, in biotically rigorous habitats of the shallow tropics, natural selection often favors the evolution of offensive weapons and attack behaviors that speed up rather than slow down already slow styles of attack, like drilling predation (Vermeij and Carlson, 2000; Herbert, 2004; Dietl et al., 2004). The use of faster, more powerful attaek techniques allows predators to spend more time in enemy-free refugia or to take additional prey, the energetie benefits of whieh eould be translated into inereased reproduction or defenses (e.g., large size, thicker shell, speed, toxins, ete.).

We hypothesize two evolutionary scenarios to explain the rare transition from predation to ectoparasitism of mollusks in the Muricidae. One hypothesis is that slow feeding on prey may be benefieial during periods of limited or unpredictable prey supply, where the benefits of mere survival outweigh the eosts of feeding slowly. During these unfavorable eonditions, selection for competitive performance is likely to be less important than selection for stress tolerance or stress avoidanee (Parsons, 1996; Stanton et al., 2000; Bijlsma and Loeschcke, 2005; but see Chesson and Huntly, 1997). Stressful abiotic conditions may, thus, stimulate the evolution of resource-eonserving traits or behaviors related to metabolic conservatism. For muricids, these environmental stresses would have to be extreme, because some muricids can survive months without feeding (Herbert, unpublished observations), and many murieids are generalist predators eapable of exploiting a wide range of prey.

This scenario is appealing on the surface, because it would also explain how a murieid predator might tolerate the potentially greater exposure to enemies during slow feedings. Places and times of low productivity and nutritional stress generally also have lower abundances and diversities of enemies (Vermeij, 1989; Bambach, 1993; Bambach et al., 2002; Valentine et al., 2002). However, this seenario contrasts markedly with the current distribution of *V. salebrosa* in the tropical eastern Pacific, which is resource rieh due to seasonal upwelling (Bemis and Geary, 1996) and where there is a relatively high abundance of prey and intense predation (Vermeij and Currey, 1980; Vermeij, 1989). This scenario also contrasts with the current distribution of its ectoparasitic congener, *V. miliaris* in the highly productive Indo-Paeific and with the aneient distributions of some fossil *Vitularia* in the productive tropical western Atlantic (reviewed by Allmon, 2001).

A second hypothesis is that in a dangerous environment, like the one in which species of *Vitularia* occur today and in which likely oeeurred in the past, ectoparasitism permits individuals to stay for long periods of time on a single prey and under a single boulder rather than to have to forage out in the open between boulders, exposed and unprotected, on a frequent basis. The exposure factor could be signifieant for *V. salebrosa*, because although the shell is relatively large, it is also remarkably thin and could be easily erushed by most durophagous predators. In a competitive environment, ectoparasitic feeding by *V. salebrosa* through small tunnels in the host shell may also reduce competitive interactions with kleptoparasites (e.g., crabs, snails) that often steal food from muricid drillers through the gaped valves of dead or dying prey.

The energetie costs of ectoparasitism, however, are still severe and probably limiting in terms of population size, growth rates, etc. Whether these costs have limited opportunities for speciation within ectoparasite lineages or opportunities for molluscan ectoparasitism to evolve more times than it has within the Muricidae should be studied further.

It should be noted, however, that muricid ectoparasitism does not involve energetic costs by necessity. For example, coralliophiline muricids that feed ectoparasitically at the margins of coral colonies benefit from the fact that there is a tendency for photosynthetic products from healthy, non-preyed-on corallites to flow towards the colony margins, which are energy sinks due to shading and competition from other corals (Oren et al., 1998). Coralliophilines also tend to feed in aggregations (Ward, 1965; Miller, 1981; Hayes, 1990: Soong and Chen, 1991), and this behavior can also induce the development of new energy sinks even away from coral colony margins (Oren et al., 1998). Still other coralliophilines insert the proboseis into polyp coelenterons to steal food rather than eat and damage the polyp, which may result in a constant high supply of food for the snail (Hayes, 1990). Coralliophilines comprise a diverse subfamily of nearly 200 living species that, as a group, is nearly the same age as the species-poor genus *Vitularia*, which can be traced back to Eocene origins in *Odontopolys* Gabb, 1860. Thus, the degree and nature of constraints of ectoparasitism may depend, in large part, on the type of host that is exploited. Ectoparasitism on large, clonal cnidarian hosts offers access to an abundant and rapidly replenishable supply of food in a way that ectoparasitism on a single bivalve or snail does not.

ACKNOWLEDGMENTS

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Unusual anatomy of the ectoparasitic muricid *Vitularia salebrosa* (King and Broderip, 1832) (Neogastropoda: Muricidae) from the Pacific coast of Panama

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ABSTRACT

The morphology and anatomy of *Vitularia salebrosa*, a muricid ectoparasitic on other mollusks, are investigated based on study of specimens from western Panama. Distinctive characters of this species include the small size of the buccal mass and radular apparatus, simplification of the odontophore muscles and diminished lateral teeth of the radula; an elongated, narrow proboscis; narrow digestive tract and a differentiable glandular region at the beginning of the posterior esophagus. These traits are consistent with adaptive specialization for an ectoparasitic life history.

INTRODUCTION

Herbert et al. (2009) have shown that *Vitularia salebrosa* (King and Broderip, 1832) is an ectoparasitic gastropod that can feed suctorially on a single molluscan host for months by drilling through the host's hell and inserting its proboscis into the host's blood supplies and organs. One of the questions raised in that study was whether and to what degree the anatomy of *V. salebrosa* has undergone adaptive specialization for an ectoparasitic lifestyle. For example, foot scars formed by *V. salebrosa* on the surface of its host's shell suggest that this ectoparasite produces mucous adhesives in its foot to help it attach itself securely to prey during feeding (Herbert et al., 2009). D'Attilio (1991) and Herbert et al. (2008) also reported the absence of a radula in 80–90% of *V. salebrosa* individuals examined. Radula loss is characteristic of the muricid subfamily Coralliophilinae, which are highly specialized ectoparasites of cnidarians.

The objective of this study is to describe for the first time the anatomy of *Vitularia salebrosa* to serve as basis for further comparisons with other muricids and contribute to a systematic revision of the genus *Vitularia* Swainson, 1840 (type species: *Vitularia miliaris* (Gmelin, 1791)).

MATERIALS AND METHODS

Specimens were observed living, followed by dissections performed on specimens immersed in 70% ethanol and observed using a stereomicroscope. Scanning electron microscopy (SEM) was used to examine the radulae in the laboratory of Electron Microscopy of the Museu de Zoologia da Universidade de São Paulo. Drawings were made with the aid of a camera lucida, and dissections were also digitally photographed. The conchological description uses the terminology of Merle (2001, 2005). Acronyms for collections cited in this paper are **MZSP**, Museu de Zoologia da Universidade de São Paulo, and **PRI**, Paleontological Research Institution, Ithaca, New York, USA.

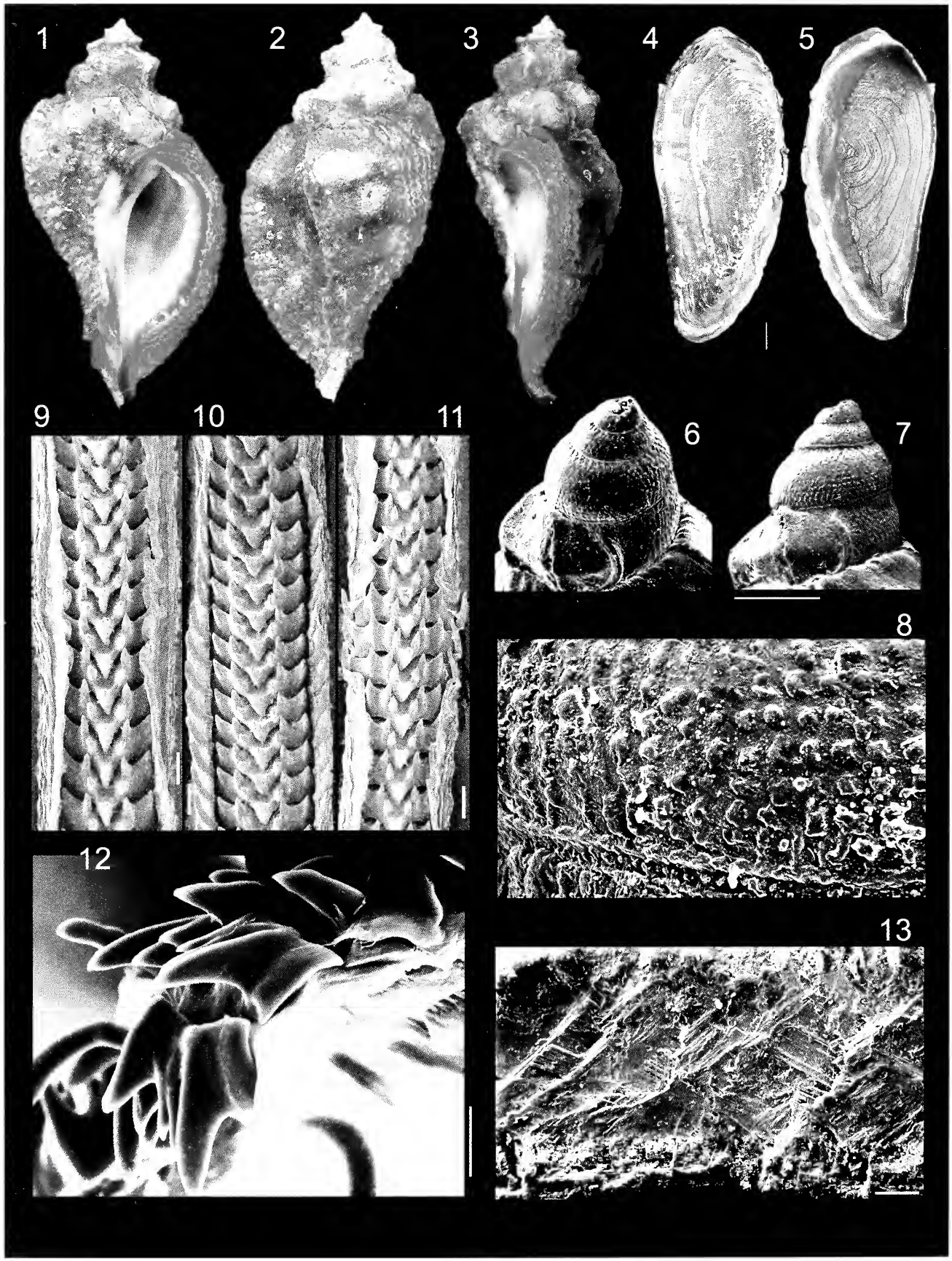
RESULTS

DESCRIPTION

Vitularia salebrosa (King and Broderip, 1832)
(Figures 1–33)

Murex salebrosus King and Broderip, 1832: 347.
Vitularia salebrosa: Keen, 1971: 536 (fig. 1040); Radwin and D'Attilio, 1976: 173–174 (figs. 114, 115; pl. 7, fig. 14); Ramírez et al., 2003: 261; Paredes et al., 2004: 214.

Shell (Figures 1–3, 6–8): Shell surface pustulose. Protoconch multispiral, with numerous granules, aligned in axial and spiral directions. Sinusigeral scar well marked. Early teleoconch whorl with P1 cord. Axial sculpture with lamellose varices. Adult teleoconch with only P1 evident. Infrasutural denticle split, eight internal denticles present, perhaps corresponding to D1 to D6 or D1 to D5 (with several split denticles). Columellar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tubercles absent. Microstructure with three shell layers; an innermost, thin aragonite layer, a thick, middle aragonite layer, and one thin, outer calcite layer (Figure 13). Complementary descriptions in Radwin and D'Attilio (1976: 173–174) and Herbert et al. (2009).

Head-Foot (Figures 14, 15, 20): Head not protruded, small (about 1/4 of adjacent width of head-foot). Tentacles stubby, broad, flat, broader basally; length about 1/3 of wider width of head-foot. Eyes dark, small, situated in middle region of outer edge of tentacles. Tentacles situated close to each other, with space between them about 1/2 the tentacular width. Rhynchostome a small, transverse slit located between and slightly ventral to tentacles. Foot large, spanning about 1/2 whorl. Anterior furrow of pedal glands exending along entire anterior edge of foot. Columellar muscle thick, about 3/4 whorl in length. Haemocoel long, slightly broader anteriorly and narrower posteriorly (Figure 20). Accessory boring organ (ABO) very narrow and relatively deep (about 1/4 of foot thickness), better developed and associated with cement gland in females (Figure 15, **fc**); sharing the same aperture.

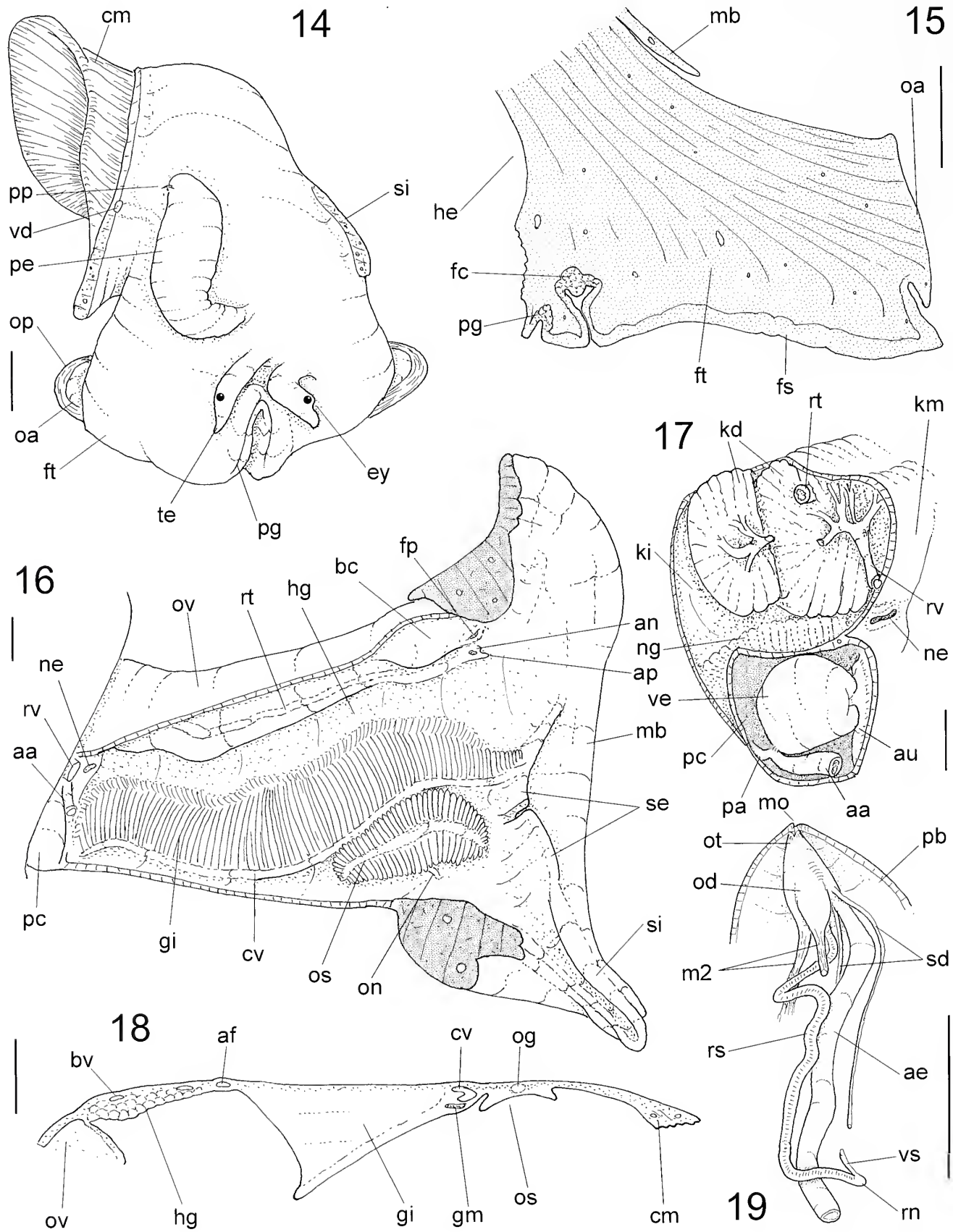
Operculum (Figures 4, 5): Suboval, filling entire aperture. Superior edge rounded; inferior edge broadly pointed; inner edge almost straight in inferior half and rounded in superior half; outer edge uniformly rounded. Outer surface opaque, mostly smooth; conspicuous scales parallel to edge in superior and inferior slopes of outer edge. Nucleus at middle level of outer margin. Attachment scar occupying about 80% of inner surface, with concentric, somewhat uniform undulations. Outer margin glossy, uniform in width (about 1/4 opercular width) along entire length of operculum.

Mantle Cavity Organs (Figures 16, 18): Mantle cavity spans about one whorl. Mantle border simple, slightly thickened. Siphon comprises about 1/3 of free portion of mantle edge width and about 1/3 whorl in length. Right edge of siphon base forming tall fold that runs parallel to mantle edge and extends approximately 1/2 width of mantle cavity (Figure 16, se); middle region of this fold tall (about 1/2 of mantle cavity height), right end of this fold diminishing gradually, becoming weaker near mantle edge. Osphradium elliptical, 1/4 mantle cavity length, 1/5 of mantle cavity roof width. Osphradium leaflets very low (about 1/4 width); tips sharply pointed, turned externally. Anterior portion of osphradium well-separated from gill. Osphradial nerve enters in middle region of osphradial ganglion (Figure 16, **on**). Ctenidial vein (efferent branchial vessel) uniformly narrow, along its length. Ctenidial longitudinal muscle covers about 3/4 of ventral surface of ctenidial vein (Figure 18, **gm**). Ctenidium elongated, spanning 85% of mantle cavity length, about 1/2 its width. Anterior end of ctenidium pointed, inserted into right surface of tall fold formed by right siphonal base. Ctenidium uniform in width along most of its length, increasing in size relatively abruptly toward the posterior margin. Posterior end of ctenidium rounded, situated close to posterior end of mantle cavity and to pericardium. Ctenidial filaments triangular, spanning ~1/2 mantle cavity height, apex central, slightly turned to right, left and right edges straight. Afferent ctenidial vessel very narrow, running along right margin of gill. Space between ctenidium and right pallial organs roughly 1/2 gill width. Hypobranchial gland thin, with uniform surface, pale-beige, covering most of area between the gill and right pallial structures. Right side of mantle cavity nearly filled by gonoducts (Figures 16, 32). Rectum very narrow, almost filiform, running along right edge of mantle cavity in young specimens, dislocated to left by gonoducts of mature specimens. Anus very small, situated at 1/4 mantle cavity length from mantle edge, with small terminal papilla (Figures 16, 32, **ap**).

Visceral Mass (Figures 26, 29): Visceral mass tapering, spanning ~2½ whorls posterior to the mantle cavity. Digestive gland pale-beige with small black spots, occupying most of the visceral mass, surrounding the stomach, extending from visceral apex to kidney-pericardium. Gonad also pale-beige, situated along the columellar surface of the digestive gland, extending from the first whorl to 1/2 whorl posterior to stomach.

Circulatory and Excretory Systems (Figure 17): Reno-pericardial region spanning ~1/3 whorl, situated at anterior margin of visceral mass, partly adjacent to the mantle cavity, roughly triangular in cross-section, broadest along right margin. Pericardium occupying ~1/3 of reno-pericardial region, just posterior to gill at anterior-left margin of visceral mass (Figures 16, 29). Auricle anterior to ventricle, connected to ctenidial vein (efferent branchial vessel) at its left-anterior side, to reno-pericardial duct along its right side; distance between connections ~1/4 adjacent whorl width. Ventricle spherical, connected to aortas at its posterior-left side. Aortas narrow, anterior aorta about twice diameter of posterior aorta, running parallel to esophagus. Kidney somewhat elliptical in outline. Renal lobe single, mostly solid, with imbricated, septum-like, transverse, glandular folds, all connected at middle region of ventral surface by longitudinal efferent renal vessel coming from haemocoel; lobe surrounding intestine-rectum transition alongside right region; color cream, surface transversally folded, filling most of kidney inner space, not connected to ventral renal surface. Nephridial gland ~1/4 width of renal lobe, triangular in section; covering entire membrane between

Figures 1–13. *Vitularia salebrosa,* shells. **1–3.** PRI 9468, apertural, dorsal and profile views, length = 40.0 mm. **4–5.** Typical operculum, outer and inner views, scale bar = 2 mm. **6–8.** SEM of Protoconch, PRI 9469. **6.** Lateral-slightly apical view. **7.** Lateral view. **8.** Detail of sculpture of penultimate whorl, scale bar = 50 µm. **9–12.** Radulae of 3 specimens, SEM. Scale bars = 20 µm. **13.** Transverse section of shell, SEM, scale = 100 µm.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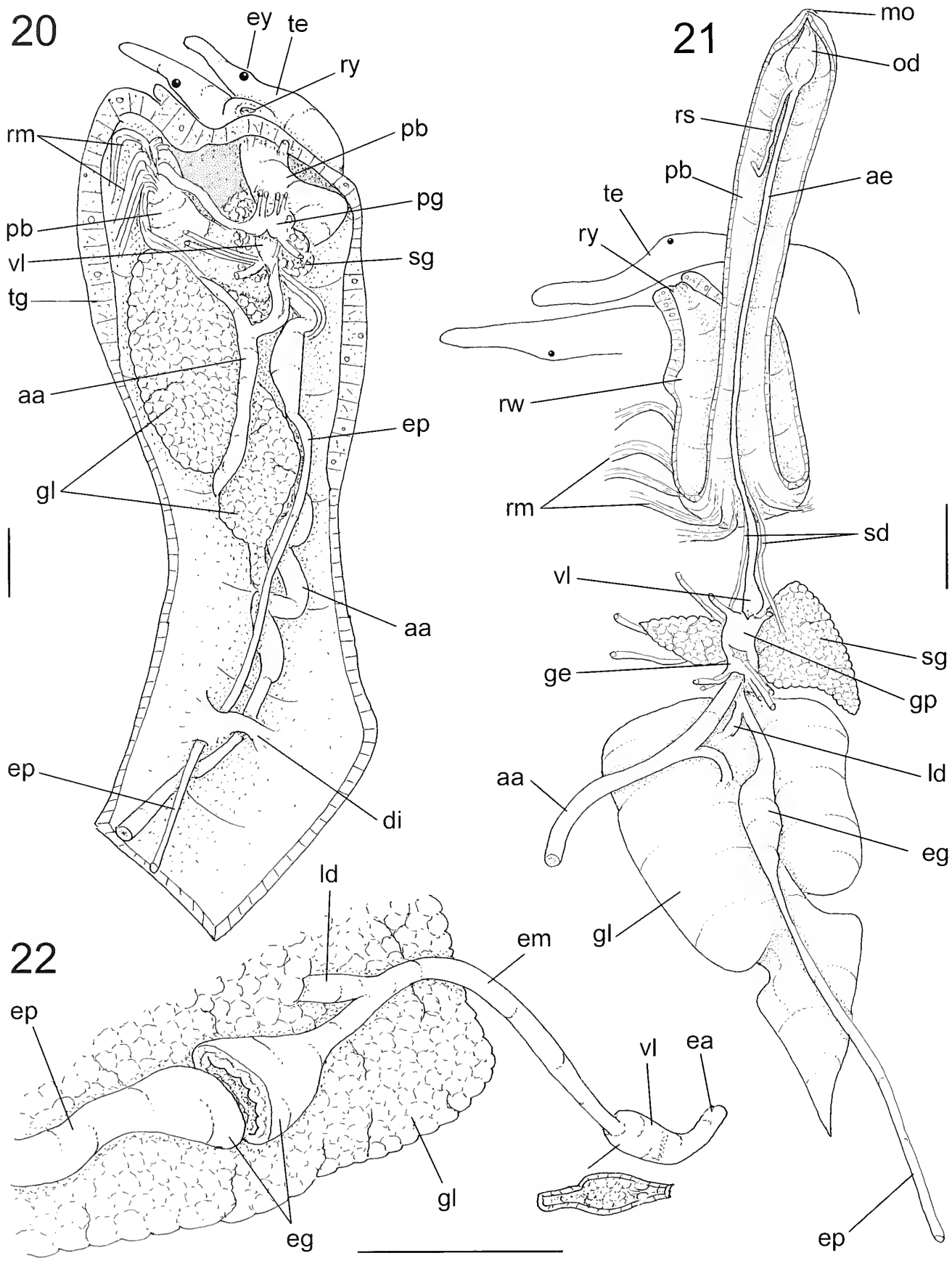
kidney and pericardium, wider dorsally. Nephrostome a small slit in kidney wall in mantle cavity (Figures 16, 17, **ne**).

Digestive System (Figures 19–26): Proboscis narrow and very long (~3 times shell length, 1/4 haemocoel width), outer walls thin, muscular (Figures 20, 21). Pairs of ventral proboscis retractor muscles (**rm**) narrow, originating in dorsal surface of foot, concentrated along right region, just ventral to head (Figure 20); closer to buccal mass, retractor muscles almost imperceptible, embedded in proboscis wall (Figure 21). Mouth transverse, narrow. Oral tube short, broad, walls weakly muscular. Dorsal folds paired, originate along dorsal, inner surface of oral tube, become more longitudinal posteriorly (Figure 23, **df**), with a narrow, smooth surface between them. Odontophore very small, ~1/15 proboscis volume, situated just posterior to mouth (Figure 21, **od**). Odontophore and buccal mass muscles (Figures 23–25): **mj**, peribuccal muscles, paired, thick layers of muscles connected along both sides of anterior-outer margin of odontophore cartilages (Figures 24, 25), embedded in dorsal wall of buccal mass; **m1**, jugal muscles, several pairs of small, short fibers connecting buccal mass with adjacent inner surface of proboscis; **m2**, pair of retractor muscles of buccal mass (retractor of pharynx), originating in ventral surface of haemocoel (dorsal surface of foot sole) at mid-length, just posterior to proboscis retractor muscles, extend anteriorly and dorsally as a pair of inconspicuous longitudinal muscles, inserting into posterior end of both odontophore cartilages; **m4**, pair of large, broad, thin, dorsal tensor muscles of radula, originating along outer surface of cartilages, surrounding **mj** origin, covering most of cartilage surface (except edge close to median line), inserting mostly into subradular membrane, and also in a small region of tissue in radular ribbon (anterior to its exposed area) (Figures 24, 25, **to**); **m5**, pair of auxiliary dorsal tensor muscles of radula, thin, originating along median edges of cartilages along their posterior quarter, running medially and anteriorly, inserting along ventral portion of radular sac, crossing odontophore (opposite to **m4** insertions in tissues on radula); **m6**, horizontal muscle, relatively thin, connecting ventral edges of both cartilages, from anterior end of cartilages, posteriorly ~60% of their length; **m11**, paired ventral tensor muscles of radula, thin, narrow, originating at median-posterior ends of odontophore cartilages, extending dorsally to **m5** origins, running anteriorly at some distance from median line, inserting along anterior surface of ventral region of subradular membrane (Figure 24). Other non-muscular odontophore structures: **br**, subradular membrane, thin, semi-transparent, strong, connecting to **m4** muscle pair at lateral and anterior edges, covering inner surface of subradular cartilage; **sc**, subradular cartilage expansions, elliptical, covering about half of exposed portion of subradular membrane within buccal cavity, bearing exposed part of radula, expanding beyond it laterally equal to the width of the radula on each side; **oc**, odontophore cartilages, flat, long, paired, about 5 times as long as wide, elliptical in outline, anterior somewhat pointed, slightly wider than rounded posterior; **to**, tissue on radula posterior to its exposed portion within buccal cavity, located inside radular sac along its region crossing odontophore, **m4** muscle pair insert into it laterally along a region ~1/10 cartilage length. Radular sac narrow (~1/5 of odontophore width), long (4 times buccal mass length) (Figure 19). Radular nucleus (odontoblast region of radular sac) slightly broad, connected to inner surface of proboscis by relatively wide vessel with thin, muscular walls (Figure 19). Radular teeth (Figures 9–12): Rachidian teeth wide, ~3/5 of radular ribbon width, chevron-like, with 7 conical, pointed, posteriorly-directed cusps that are not aligned; central cusp taller, at a greater angle to ribbon than remaining, lateral cusps, which are situated nearly on the same plane; lateral edges of rachidian teeth broad, flattened. Lateral teeth paired, very narrow, ~1/8 of rachidian teeth width, equal to rachidian teeth in height (L/W ~5), weakly curved; bases wider, inserted into subradular cartilage close to proximal region of rachidian teeth lateral edge; tip sharply pointed, turned posteriorly. Salivary glands just posterior to valve of Leiblein, anterior to nerve ring (Figure 21, **sg**), occupying ~1/8 of haemocoel volume. Salivary gland ducts very narrow; gradually become embedded in anterior esophagus wall anterior to valve of Leiblein (Figure 21). Accessory salivary glands absent. Anterior esophagus narrow, long (Figure 21), equal in length to proboscis, inner surface smooth, with pair of low, narrow longitudinal folds in anterior region

Figures 14–19. *Vitularia salebrosa* anatomy. **14.** Head-foot, male, frontal view. **15.** Foot, female, longitudinal section in median line. **16.** Mantle cavity roof, female, ventral view, transversal section in fold of right base of siphon artificially done. **17.** Reno-pericardial region, ventral view, ventral wall of pericardium and part of kidney removed, posterior region of renal lobe partially deflected. **18.** Mantle cavity roof, female, transversal section in middle level of osphradium. **19.** Distal region of foregut, ventral-right view, distal portion of proboscis also shown. Scale bars = 2 mm. Abbreviations: **aa,** anterior aorta; **ae,** anterior esophagus; **af,** afferent branchial vessel; **an,** anus; **ap,** anal papilla; **au,** auricle; **bc,** bursa copulatrix; **bv,** blood vessel; **cm,** columellar muscle; **ev,** ctenidial vein; **ey,** eye; **fc,** female cement gland plus boring organ; **fp,** female pore; **fs,** foot sole; **ft,** foot; **gi,** gill; **gm,** gill longitudinal muscle; **he,** haemocoel; **hg,** hypobranchial gland; **kd,** kidney dorsal lobe; **ki,** kidney chamber; **km,** membrane between kidney and mantle cavity; **m2,** buccal mass and odontophore muscles; **mb,** mantle border; **mo,** mouth; **ng,** nephridial gland; **ne,** nephrostome; **oa,** opercular pad; **od,** odontophore; **og,** osphradium ganglion; **on,** osphradium nerve; **op,** operculum; **os,** osphradium; **ot,** oral tube; **ov,** pallial oviduct; **pa,** posterior aorta; **pb,** proboscis; **pc,** pericardium; **pg,** pedal gland furrow; **pp,** penis apical papilla; **rs,** radular sac; **rt,** rectum; **rv,** efferent renal vessel; **sd,** salivary duct; **se,** fold of siphonal base; **si,** siphon; **te,** cephalic tentacle; **vd,** vas deferens; **ve,** ventricle; **vs,** blood vessel.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(Figure 23, **df**). Valve of Leiblein slightly wider than surrounding esophagus, anterior half conical, posterior half rounded (Figure 22). Internally, valve anterior with a tall cylindrical fold, with relatively short cilia directed posteriorly (Figure 22). Remaining portions of valve of Leiblein entirely covered by inner, thick whitish glandular layer. Middle esophagus about same diameter as anterior esophagus (Figure 21); inner surface smooth, simple. Gland of Leiblein occupying ~1/3 of haemocoel volume, broad, flat anteriorly, gradually narrowing posteriorly, becoming very narrow, sharply pointed (Figures 20, 21). Duct of gland of Leiblein broad, situated at some distance from anterior end of gland. Posterior esophagus narrow, equal in length to anterior esophagus (Figures 21, 26), with a broadly expanded glandular region (Figures 21, 22, **eg**) situated beneath gland of Leiblein, posterior to duct of gland of Leiblein (by ~1/10 posterior esophagus length). Glandular lining of this region of posterior esophagus about twice as thick as esophageal wall. Stomach a simple curve (Figure 26), equal in width to esophagus, located about 1/3 whorl posterior to kidney, embedded in digestive gland. Inner surface smooth, simple. Duct to digestive gland single, joining stomach in posterior gastric curve, about equal in diameter to intestine. Intestine as wide as esophagus, nearly straight, running anteriorly along right region of visceral mass, passing through ventral region of renal lobe (Figure 17). Digestive gland, rectum and anus described above.

Male Genital System (Figures 14, 27–29, 36): Visceral vas deferens running from testis along columellar surface of visceral mass to intensely coiled seminal vesicle located on mid-ventral region of last whorl of visceral mass, comprising ~1/4 of mass of adjacent region of visceral mass (Figure 29, **sv**). Vas deferens narrow, simple, straight, running along ventral wall of kidney, exiting into mantle cavity along its middle-posterior edge (Figure 29, **vd**). Pallial vas deferens strongly convoluted for 1/4 of mantle cavity length along right-ventral edge of mantle cavity, connecting to posterior end of prostate gland. Prostate gland ~1/4 of mantle cavity length, ~1/10 its width (Figure 28, **pt**), with glandular, iridescent, walls narrowing anteriorly, lacking clear separation with remaining anterior vas deferens, which crosses to pallial floor at level of anus, winding sigmoidally to base of penis (Figures 14, 27, 36); pallial vas deferens entirely closed (tubular) (Figure 27, **vd**). Penis broadest medially (1/3 penis length), somewhat flattened, occupies ~1/6 mantle cavity volume, curved at base; apical region narrowing abruptly, rounded; apical papilla narrow, ~1/8 of penis length, located within protective apical chamber that occupies ~1/10 of penis volume (Figure 27). Penis duct (~1/6 of penis width) runs along penis axis, strongly coiled at mid-length (Figure 27), narrowing at papilla base, opening at papilla tip.

Female Genital System (Figures 16, 32, 33): Visceral oviduct relatively wide, entering left posterior region of albumen gland (Figure 32). Pallial oviduct massive (Figure 16), (~2/3 length, ~1/3 width of mantle cavity). Albumen gland spherical, flattened, walls thick, white, about ~1/4 pallial oviduct length; lumen broad and flat, continuous with that of capsule gland. Capsule gland long (~2/3 pallial oviduct length), slightly narrower than, and anterior to, albumen gland; walls thick, glandular, pale beige in color; lumen broad and flat (Figure 32). Anterior region of capsule gland with thinner walls, forming vaginal atrium (Figures 32, 33, **vg**). Bursa copulatrix elliptical, ~1/6 pallial oviduct length, situated on ventral, left side of anterior end of pallial oviduct. Bursa walls thick, longitudinally folded. Capsule gland and bursa copulatrix ducts converge anteriorly to form small genital papilla (Figure 33, **fp**), located within small chamber.

Central Nervous System (Figures 20, 21, 30, 31): Nerve ring located in anterior region of haemocoel, at proboscis base (Figures 20, 21). Nerve ring volume approximately 1/20 that of haemocoel. Ganglia highly concentrated and difficult to separate. Cerebral and pleural ganglia paired, totally fused. Pedal ganglia paired, as large as cerebro-pleural ganglia, broadly connected to each other and to remaining main ganglia. Sub-esophageal ganglion close to nerve ring, about half the size of a pedal ganglion. Statocysts not found.

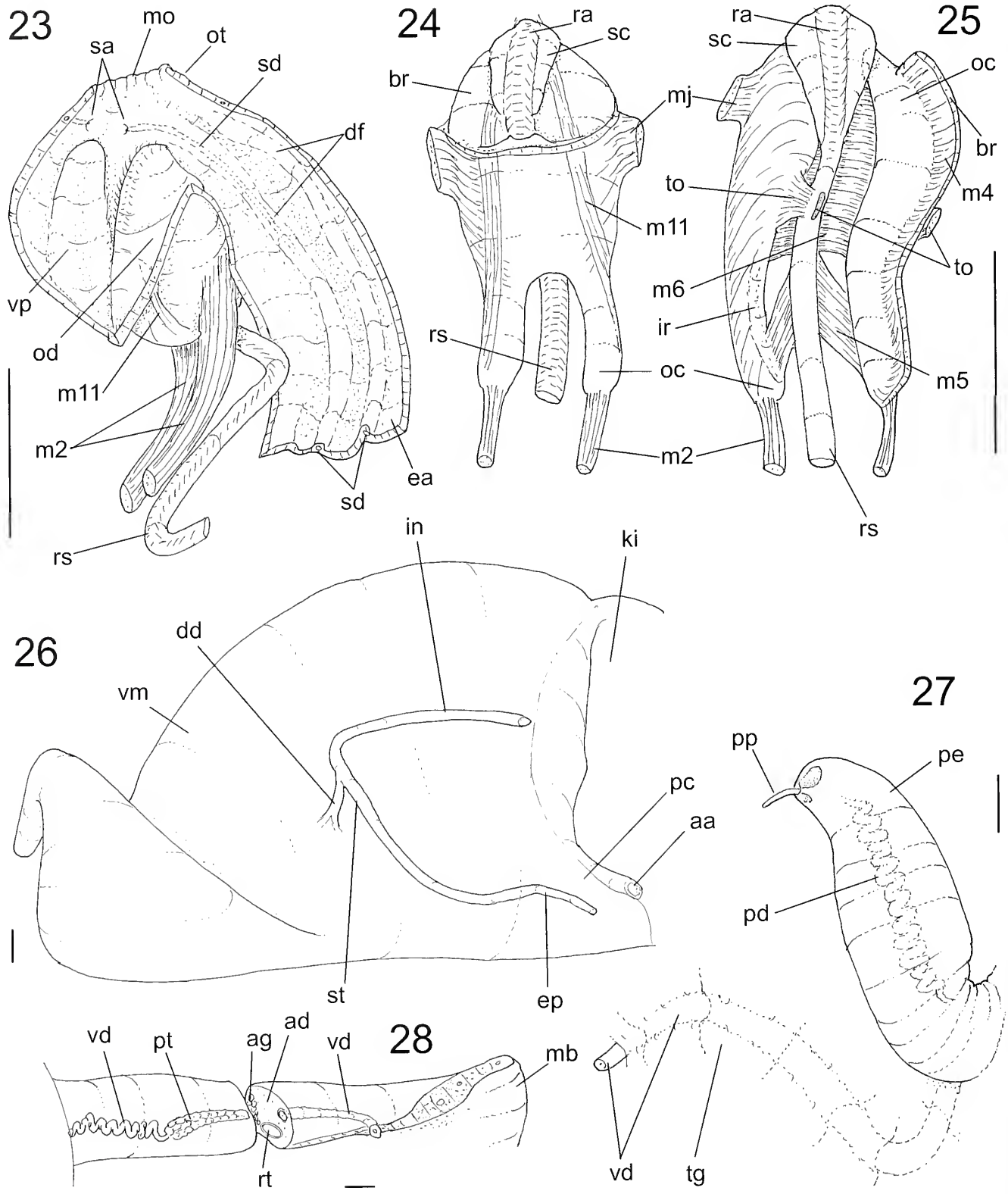
Measurements (in mm): MZSP 63824: ♀1: 64.7 by 33.1; ♂3: 47.4 by 25.8; MZSP 64213 ♀1: 62.1 by 28.4; PRI 9468: 40.0 by 22.3 (Figs. 1–3).

Geographic Distribution: Baja California to Peru.

Habitat: Under rocks, intertidal and subtidal.

Material Examined: W. PANAMA (Gulf Panama): Panamá City, MZSP 10173, 1 shell; Chumical Arerajan, Chumical Bay Playa, 08°53′08.8″ N, 79°38′37.7″ W, MZSP 63824, 1♂, 2♀ (Simone col., 29 Jan. 2006); Venado Island, 08°52′48.6″ N, 79°35′36.9″ W, MZSP 64213, 4♂, 2♀ (Simone col. 30 Jan. 2006), MZSP 77671,

Figures 20–22. *Vitularia salebrosa* anatomy. **20.** Head and haemocoel, ventral view, foot and columellar muscle removed, inner structures as in situ. **21.** Foregut removed, ventral view, some adjacent structures also shown. **22.** Detail of foregut region between valve and gland of Leiblein, ventral view, with detail of valve opened longitudinally, a transversal section artificially done in proximal region of posterior esophagus. Scale bars = 2 mm. Abbreviations: **aa,** anterior aorta; **ae,** anterior esophagus; **di,** diaphragm-like septum; **ea,** anterior esophagus; **eg,** gland of posterior esophagus; **em,** middle esophagus; **ep,** posterior esophagus; **ey,** eye; **ge,** sub-esophageal ganglion; **gl,** gland of Leiblein; **gp,** pedal ganglion; **ld,** duct of gland of Leiblein; **mo,** mouth; **od,** odontophore; **pb,** proboscis; **pg,** pedal gland furrow; **rm,** proboscis retractor muscle; **rs,** radular sac; **rw,** rhynchodeal wall; **ry,** rhynchostome; **sd,** salivary duct; **sg,** salivary gland; **te,** cephalic tentacle; **tg,** integument; **vl,** valve of Leiblein.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3♀, 83792, 7 specimens (Simone col. 01/ii/2006), PRI 9468, 2 specimens (Figures 1–3, 6–8). Las Perlas Archipelago, 08°21′27.7″ N, 78°50′28.7″ W, MZSP 78481, 2 specimens (Simone col. 4 Feb. 2006). COSTA RICA: Joce Beach, PRI 9469, 1 specimen. ECUADOR: Manabi; Isla Salango, MZSP 67408, 1 shell, MZSP 69597, 12 shells (Coltro col. Mar. 2003).

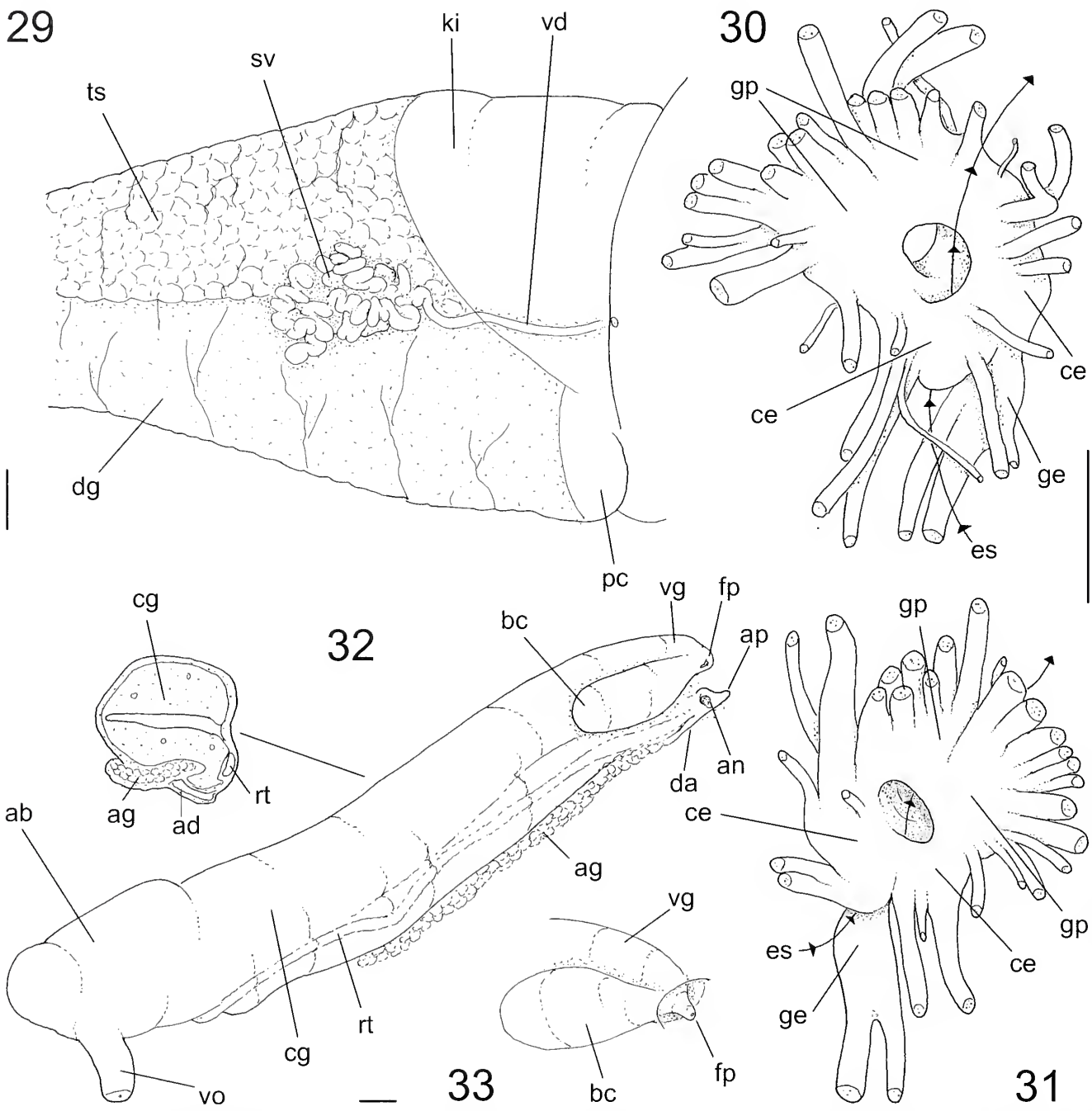
DISCUSSION

The anatomy of *Vitularia salebrosa* is comparable to that described for numerous muricids (e.g., Harasewych, 1984; Kool, 1987, 1993a, b; Ball et al., 1997; Tan and Sigurdsson, 1996; Tan, 2003; Simone, 2007), and shares features characteristic of the family, among them a mantle border that closely surrounds the siphon, an accessory boring organ, and an anal papilla. However, several aspects of the morphology of *V. salebrosa* appear to be unique. These include: (1) a tall, septum-like fold at the right base of the siphon (Figure 16, **se**); (2) an elongated proboscis (Figure 21) (muricids normally bear a well-developed, but shorter proboscis); (3) a small and simple buccal mass, particularly the odontophore (Figure 19), with small **m6** and retractor muscle pairs **m5**, and the lack of a muscular connection in the radular sac (Figures 24, 25); (4) a relatively small pair of lateral teeth on the radula (Figures 9–12); (5) a digestive system that is simplified and reduced in diameter (Figures 21, 22, 26), particularly the stomach, which is reduced to a simple, inconspicuous curve that is joined by the duct of the digestive gland; (6) a reduced valve of Leiblein that lacks a transverse furrow, or by-pass, along its length (Figure 22, **vl**); (7) a mid-esophagus that is simple, rather than glandular as in most muricids (Figures 21, 22, **eg**); (8) an anal gland (Figure 32, **ag**) that is unusually elongated; (9) a prostate gland that is relatively small, with a long, convoluted vas deferens in the mantle cavity (Figure 28); (10) a penis with a terminal papilla (common in muricids) that is protected by an unusual terminal chamber. Similarly, the female genital pore is also protected in a small, hollow chamber (Figure 33), while the remainder of the pallial oviduct is normal for the family; and (11) a central nervous system, or nerve ring, that is more concentrated than usual (Figures 30, 31) (normally, the muricid nerve ring is slightly longer dorso-ventrally, with a clearer separation between the pedal ganglia and the remaining ganglia).

This study did not confirm the findings of D'Attilio (1991) and Herbert et al. (2008), who reported that as many as 80–90% of animals studied lacked a radula. All animals dissected in this analysis (n=23) possessed a radula. The different results obtained herein can be interpreted several ways. Herbert et al. (2009) found that ectoparasistic interactions between an individual *Vitularia salebrosa* and a single molluscan host can last many months and possibly as long as a year. They also found that interactions with new oyster prey appear to start at the same time each year. Because *V. salebrosa* requires a radula to initiate the interaction by drilling a feeding hole, it may be that a functioning radula is present sporadically, and perhaps seasonally (see also Herbert et al., 2009). An alternative explanation is that the radula was destroyed by the cleaning process used in past work, which involved dissolving tissues either in concentrated potassium hydroxide, or in a bleach-like solution. It is not clear, however, why this technique works so well for other muricids but would fail consistently for *V. salebrosa*, unless its radula is sometimes non-mineralized. It is also possible that the long proboscis was accidentally amputated by collectors vigorously pulling the feeding animal from its host. Each of these explanations can be easily tested in future work.

Differences in the digestive system between *Vitularia salebrosa* and other muricids, particularly the simplification and reduction in diameter of its gut, are compatible with an ectoparasitic mode of life. On the other hand, as the digestive system is complete in *V. salebrosa*, it is possible to infer that parasitism is not obligatory, and that normal predatory behavior can also occur. The muricid subfamily Coralliophilinae is known to include species that are ectoparasitic on cnidarians, yet there are few parallels between the anatomy of coralliophilines and that of *V. salebrosa*. A striking aspect of the anatomy of *V. salebrosa* is the highly reduced diameter of its digestive tract, although it has retained a fully functional buccal mass and odontophore. In contrast, coralliophilines have suffered severe atrophy of the anterior portion of the digestive system, particularly the buccal mass, including the total loss of the odontophore, radula, and related structures. However, the remaining portions of the digestive system in coralliophines are relatively similar to those of other muricids.

Figures 23–28. *Vitularia salebrosa* anatomy. **23.** Buccal mass, right view, esophagus and ventral region opened longitudinally, way of right salivary duct partially shown, radular sac only partially shown. **24.** Odontophore, ventral view. **25.** Same, ventral view, left structures (right in Figure) partially deflected, superficial layer of tissues removed. **26.** Visceral partially uncoiled showing topology of midgut, ventral view, topology of some portions of reno-pericardial structures also shown. **27.** Penis and adjacent region of head, dorsal view, some penial inner structures artificially shown. **28.** Detail of right region of mantle cavity, male, ventral view, with focus on genital structures, a transverse section through middle region. Scale bars = 1 mm. Abbreviations: **aa,** anterior aorta; **ad,** adrectal sinus; **ag,** anal gland; **br,** subradular membrane; **dd,** duct to digestive gland; **df,** dorsal inner folds of buccal mass; **ea,** anterior esophagus; **ep,** posterior esophagus; **in,** intestine; **ir,** insertion of m4 radular sac; **ki,** kidney chamber; **m1–m11,** buccal mass and odontophore muscles; **mb,** mantle border; **mj,** peri-oral muscles; **mo,** month; **oc,** odontophore cartilage; **od,** odontophore; **ot,** oral tube; **pe,** pericardium; **pd,** penis duct; **pe,** penis; **pt,** prostate gland; **ra,** radula; **rs,** radular sac; **rt,** rectum; **sa,** salivary duct aperture; **sc,** subradular cartilage; **sd,** salivary duct; **st,** gastric region; **tg,** integument; **to,** tissue connecting m4 with radular sac; **vd,** vas deferens; **vm,** visceral mass; **vp,** ventral platform of buccal mass.



Figures 29–33. *Vitularia salebrosa* anatomy. **29.** Anterior region of visceral mass, male, ventral view. **30.** Nerve ring, ventral view, with indication of esophagus topology (successive arrows). **31.** Same, dorsal view. **32.** Pallial oviduct, ventral view, some adjacent structures also shown, a transversal section of indicated region also revealed. **33**. Detail of anterior region of pallial oviduct, ventral view, with inner terminal genital papilla protruded. Scale bars = 1 mm. Abbreviations: **ab,** albumen gland; **ad,** adrectal sinus; **ag,** anal gland; **an,** anus; **ap,** anal papilla; **bc,** bursa copulatrix; **ce,** cerebro-pleural ganglion; **cg,** capsule gland; **da,** anal gland duct; **es,** esophagus; **fp,** female pore; **ge,** sub-esophageal ganglion; **gp,** pedal ganglion; **ki,** kidney chamber; **rt,** rectum; **sv,** seminal vesicle; **ts,** testis; **vd,** vas deferens; **vg,** vaginal atrium; **vo,** visceral oviduct.

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The distribution of precursors and biosynthetic enzymes required for Tyrian purple genesis in the hypobranchial gland, gonoduct, and egg masses of *Dicathais orbita* (Gmelin, 1791) (Neogastropoda: Muricidae)

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ABSTRACT

The biosynthetic origin of Tyrian purple in the adult hypobranchial gland and egg masses of the Muricidae is mknown. Histochemistry and mass spectrometry were employed to determine the distribution of biosynthetic components essential for Tyrian purple precursor synthesis within the hypobranchial gland, gonoduct, egg masses, and larvae of *Dicathais orbita*. Histochemical correlations suggest that *de novo* synthesis of the prochromogen, tyrindoxyl sulphate, not only occurs within the hypobranchial gland, but also within the gonoduct, eapsule, intracapsular fluid, and encapsulated larvae. The coineidence of tyrindoxyl sulphate and arylsulphatase in the capsule and albumen glands, along with the capsule wall and intraeapsular fluid, suggest that the biosynthetic eomponents required for Tyrian purple synthesis are introduced during eapsule formation. Overall it appears that the egg mass natural produets of the Muricidae arise from a maternal souree.

Additional keywords: Bromoperoxidase, arylsulphatase, tyrindoxyl sulphate, capsule, intracapsular fluid, vitellus, natural products

INTRODUCTION

Tyrian purple is an aneient dye of religious and royal significance (Reinhold, 1970) obtained exclusively from hypobranchial gland secretions of muricid mollusks (Cooksey, 2001). Although the east Mediterranean Tyrian purple industry of the 13th Century B.C. once flourished (McGovern and Michel, 1985), traditional dye production has now been all but abandoned (Naegel and Cooksey, 2002). Nevertheless, the historical importance of Tyrian purple has prompted considerable investigation into the chemical composition and formation of this dye.

In 1909, Friedländer elucidated the dominant dye pigment as 6,6'-dibromoindigo (Figure 1, **6**). Much later, Baker and Sutherland (1968) isolated the proehromogen, tyrindoxyl sulphate (Figure 1, **2**) from the Australian muricid, *Dicathais orbita* (Gmelin, 1791). Proehromogen hydrolysis by arylsulphatase (Dubois, 1909; Baker and Sutherland, 1968) and subsequent oxidation and dimerization generates a suite of brominated intermediate dye precursors (Figure 1, **3–5**) (Cooksey, 2001). Of these, tyriverdin (Figure 1, **4**) is photolytically eleaved to yield the pigment 6,6'-dibromoindigo (McGovern and Michel, 1990; Cooksey, 2001). Depending on prochromogen composition, 6,6'-dibromoindirubin, monobrominated indoles and indirubins, indigo and indirubin may also be formed (Wouters and Verhecken, 1991; Wouters, 1992; Koren, 1995; Cooksey, 2001; Cooksey and Withnall, 2001; Karapanagiotis and De Villemeruil, 2006; Westley and Benkendorff, 2008). Despite the wealth of information available on dye genesis from indoxyl sulphate precursors, few investigations have foeused on the biosynthetic origin of proehromogens and the signifieanee of this biosynthetic pathway.

Seeondary metabolite synthesis typically oeeurs through the modification of primary metabolic pathways. Indoles are believed to arise from the essential amino aeid tryptophan (Figure 1, **1**) (Fox, 1983, Verhecken, 1989; Zinderman, 1990). Indeed, storage of tryptophan has been reported within murieid hypobranchial glands where Tyrian purple genesis is known to occur (Bolognani-Fantin and Ottaviani, 1981; Srilakshmi, 1991; Naegel and Aguilar-Cruz, 2006). Among other enzymatie conversions, tryptophan must then be brominated (Figure 1) to produee the prochromogen tyrindoxyl sulphate (Westley et al., 2006). Bromoperoxidase aetivity has been detected in hypobranchial extracts of the murieid *Hexaplex trunculus* (Linnaeus, 1758) (Jamun and Coe, 1987), whieh provides evidence for preeursor bromination and hence, *de novo* prochromogen synthesis.

Early observations by Aristotle in ~350 B.C. (Peek, 1970) and Pliny the Elder in 1st eentury A.D. (Bailey,

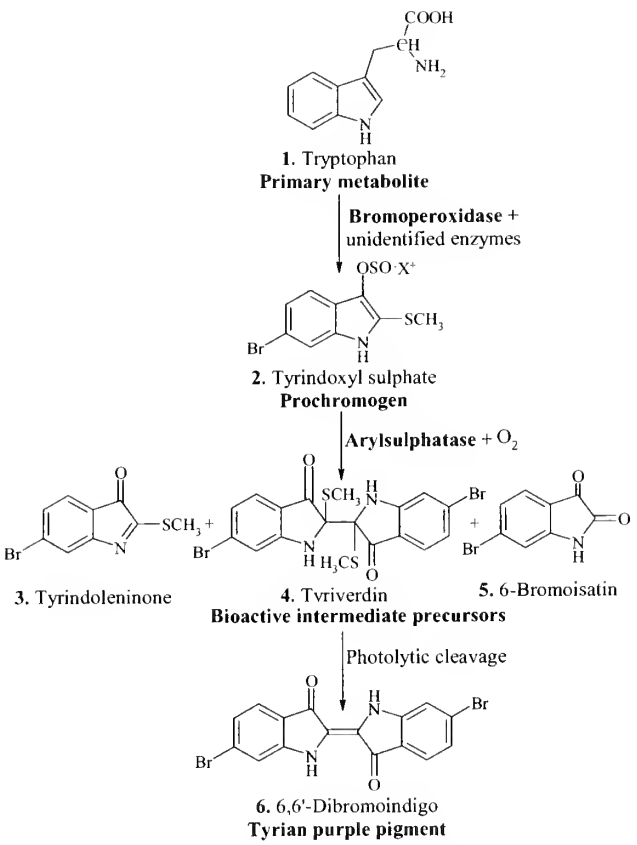

Figure 1. The proposed biosynthetic pathway to Tyrian purple from tryptophan in the muricid *Dicathais orbita* (adapted from Westley et al. 2006).

1929) indieated a link between Tyrian purple genesis and reproduction (Westley et al., 2006; Westley and Benkendorff, 2008). This association was overlooked until Tyrian purple and intermediate precursors were reeently isolated from muricid egg masses (Palma et al., 1991; Benkendorff et al., 2000, 2001, 2004). Subsequent observations reported deep red pigmentation in the gonoduct of *Dicathais orbita* (Gmelin, 1791) (Benkendorff et al., 2004) and mass spectroscopic analysis confirmed the presenee of Tyrian purple and its preeursors (Westley and Benkendorff, 2008). Although these findings imply a fundamental role for these seeondary metabolites in the reproduetion and eneapsulated development of the Murieidae, the capaeity for biosynthesis outside the hypobranchial gland remains unknown.

It is currently assumed that the compounds in egg masses arise through maternal investment during eapsule formation. However, larvae may possess the capacity to synthesize precursors *de novo*. Natural product biosynthesis has been suggested to commenee at an early larval stage in some nudibranch species (Avila, 2006). Non-viable murieid larvae are known to develop purple pigmentation (St. Amant, 1938; Gallardo, 1973; Spight, 1977; Peehenik, 1982; Roller and Stiekle, 1988; Naegel, 2004), whieh implies relevant biosynthetie competence.

This investigation aims to provide new information on the coneurrent distribution of the biosynthetic constituents essential for Tyrian purple synthesis in the hypobranehial gland, gonoduct, and egg masses of *Dicathais orbita*. These compounds and enzymes include tryptophan, bromoperoxidase, tyrindoxyl sulphate, and arylsulphatase. Overall, it is hoped these findings will highlight potential sites of prochromogen and Tyrian purple genesis and establish the importance of these secondary metabolites in muricid reproduction and larval development.

MATERIALS AND METHODS

A total of 27 female *D. orbita* specimens and 15 separately spawned egg masses were sampled from the Fleurieu and Eyre Peninsulas of South Australia. The pallial gonoduct and hypobranehial gland of 12 specimens, and the egg capsules and embryos from capsule glands were fresh-frozen cryostat sectioned (15m). Transverse sections were stained with the acid-hydrolysis method for tyrindoxyl sulphate adapted from Baker and Duke (1976), the bromo-phenol red method for bromoperoxidase modified from Krenn et al. (1989) and Wever et al. (1991) (Westley, 2008), and the post-coupling method for arylsulphatase (Rutenburg et al., 1952).

Gonoducts from 12 females, and eapsules from 9 egg masses were fixed in 10% neutral-buffered formalin and paraffin embedded. Transverse sections (5m) were stained with the p-DMAB-nitrite method (Adams, 1957) to determine sites of tryptophan storage. Cryostat and paraffin sections were also stained with Haematoxylin and Eosin (Thompson, 1966), Toluidine Blue (Kramer and Windrum, 1954) and Periodie Aeid Schiff (McManus, 1946) for morphological and bioehemical comparisons.

Tyrindoxyl sulphate distribution was determined by liquid chromatography-mass spectrometry (LC-MS). Hypobranchial, albumen, and capsule glands were excised from three females and capsules sampled from 12 egg masses. Adult tissues and separate capsule constituents (eapsule wall, intracapsular fluid and larvae) were extracted in dimethyl formamide (DMF) and analyzed according to Westley and Benkendorff (2008) by high performanee-liquid chromatography (Waters Alliance) coupled to a mass spectrometer (MS, Micromass, Quatro micro™). Tyrindoxyl sulphate was identified by registration of expeeted mass and isotopie elusters in mass spectra (Westley and Benkendorff, 2008).

RESULTS

The distribution of Tyrian purple precursors and biosynthetie enzymes required for natural produet synthesis within the female hypobranchial gland, gonoduct, and egg capsule eonstituents are summarized in Table 1. Tryptophan was detected by positive p-DMAB-nitrite staining (Figures 2–3) within the hypobranchial gland,

Table 1. The distribution of precursors and enzymes required for Tyrian purple synthesis in the female hypobranehial gland, gonoduct and egg masses of *D. orbita*. +, presence; – absence; IF, intracapsular fluid; **NA**, not attainable.

			Gonoduct		Egg mass		
Compound/ enzyme	Technique	Hypobranchial gland	Albumen gland	Capsule gland	Capsule	IF	Larval vitellus
Tryptophan	Histoehemistry	+	+	+	+	+	+
Bromoperoxidase	Histoehemistry	+	NA	+	+	+	+
Arylsulphatase	Histoehemistry	+	+	+	+	–	+
Tyrindoxyl sulphate	Histoehemistry	+	–	+	+	–	+
	LC-MS	+	+	+	+	+	+

gonoduet, egg capsule walls, intracapsular fluid and larval vitellus (Table 1). As indieated by bromophenol-red staining (Figures 4–5), bromoperoxidase displayed an identieal distribution (Table 1), although the distribution of bromoperoxidase in albumen gland tissue was not aequired due to problematic posterior gonoduet seetioning. Arylsulphatase was loealized within all adult (Figures 6–7) and larval tissues (Table 1) examined and the eapsule wall (Figure 6), but not the intraeapsular fluid (Table 1). Enzyme aetivity was generally of high activity in the capsule gland (Figure 6) and low activity in the albumen gland, capsules (Figure 6), and larvae. LC-MS revealed the presence of tyrindoxyl sulphate within the hypobranehial, albumen and capsule glands of *D. orbita* specimens, and all capsule constituents including larvae (Table 1). Prochromogen concentration was below the detectable limit by histochemical techniques in the albumen gland and intraeapsular fluid (Table 1).

DISCUSSION

Coincidence of tryptophan, bromoperoxidase, and tyrindoxyl sulphate in the hypobranchial gland of *Dicathais orbita* (Table 1) confirms prochromogen synthesis from the primary metabolite, tryptophan. These findings expand on the known oecurrenee of bromoperoxidase activity in hypobranchial gland homogenates of *Hexaplex trunculus* (Jannun and Coe, 1987) and provide further evidence for the *de novo* synthesis of brominated indoles in the Muricidae. Detection of these biosynthetie constituents within the capsule gland (Table 1) indieates that prochromogen synthesis is also possible within the muricid gonoduct. The capsule gland functions in the deposition of capsule laminae (Fretter, 1941; D'Asaro, 1988) and correlations between capsule gland and eapsule biochemistry (Table 1) confirm the introduction of tyrindoxyl sulphate and the biosynthetie componcnts for prochromogen synthesis during capsule formation. The presence of both tyrindoxyl sulphate and arylsulphatasc in capsule walls is supported by previous reports of purple pigmentation in capsules of Murieidae (Benkendorff et al., 2004). Overall, these findings provide a means of incorporating natural products into capsules and eliminatc the need to transfer precursors from the hypobranchial gland as previously suggested (Westley et al., 2006).

The eoncurrenee of arylsulphatase and tyrindoxyl sulphate within the capsule gland (Table 1) suggests that intermediate preeursor and dye genesis also oceurs within this gland. This is supported by detection of brominated indoles in *Dicathais orbita* eapsule gland extracts by Westley and Benkendorff (2008). Arylsulphatase was also found to eoineide with tyrindoxyl sulphate in the albumen gland (Table 1), which highlights this gland as another prospeetive site for preeursor synthesis. However, as tyrindoxyl sulphate was only deteetable by mass spectrometry (Table 1), proehromogen concentration must be comparatively low. This is consistent with the intracapsular fluid of *D. orbita* capsules (Table 1), whieh is thought to originate in the albumen gland in some Muricidae speeies (D'Asaro, 1988). Low proehromogen coneentrations eoupled with low arylsulphatase aetivity may also explain why bioactive intermediates were not previously reported in autolyzed albumen tissues (Westley and Benkendorff, 2008). Overall, the limited biosynthetic capaeity of the albumen gland suggests it is unlikely to contribute significant concentrations of brominated indoles to *D. orbita* egg masses.

In eomparison to the intracapsular fluid, larval vitellus was found to contain all the biosynthetic components required for Tyrian purple genesis (Table 1). This is eonsistent with previous reports of intermediate preeursors and Tyrian purple in muricid egg capsule extraets (Palma et al., 1991; Benkendorff et al., 2000, 2001). Muricid embryos are largely eomposed of nutritive vitellus (Roller and Stickle, 1988; Naegel, 2004). These yolk granules are synthesized by ovarian folliele eells and ooeytes (Martel et al., 1986; Amor et al., 2004), and eonsumed over the eourse of development (González and Gallardo, 1999). As tryptophan must be derived from the diet (Crawford, 1989; Bentley, 1990; Hermann et al., 1992), it is likely that the ovary eontributes tryptophan to yolk granules during vitellogenesis. In the ease of bromoperoxidase, arylsulphatase and possibly tyrindoxyl sulphate, it is unclear whether these originate from follicle eells or the ooeyte.

The findings of this investigation strongly indieate that bioaetive intermediate precursors in the egg masses of *D. orbita* are synthesized within the eapsule wall, larval vitellus and, to a lesser extent, the intracapsular fluid, from biosynthetie components of maternal origin. As the caenogastropod pallial gonoduct evolved from an

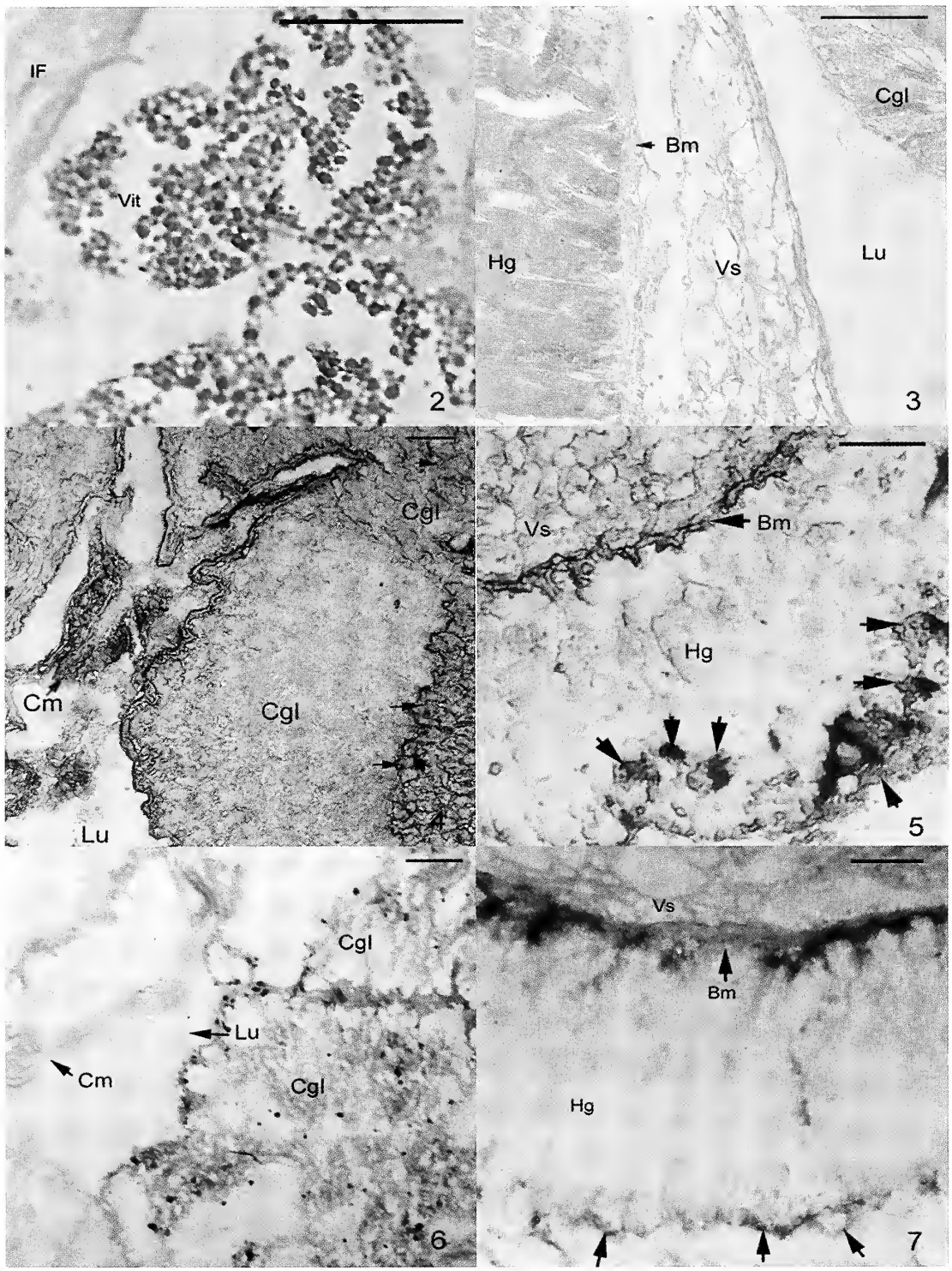

Figure 2–7. *Dicathais orbita.* Transverse histological sections. **2.** Encapsulated larvae, showing tryptophan distribution evidenced by blue p-DMAB-nitrite staining within the vitellus (**Vit**) and intracapsular fluid (**IF**). **3.** Tryptophan distribution evidenced by blue p-DMAB-nitrite staining within the capsule (**Cgl**) and hypobranchial gland (**Hg**). **4.** Capsule gland containing a partially formed egg capsule. Bromoperoxidase activity (**arrows**) indicated by bromophenol-blue staining of capsule material (**Cm**). **5.** Hypobranchial gland, showing bromoperoxidase activity (**arrows**) evidenced by bromophenol-blue staining. **6.** Capsule gland, showing arylsulphatase activity (**arrows**) displayed by red (= low levels) staining of capsule material. **7.** Hypobranchial gland, showing arylsulphatase activity (**arrows**) displayed by purple (= high levels) staining. Abbreviations: **Bm**, basement membrane; **Cgl**, capsule gland; **Cm**, capsule material; **Hg**, hypobranchial gland; **IF**, intracapsular fluid; **Lu**, lumen; **Vit**, larval vitellus; **Vs**, vascular sinus. Scale bars = 100 μm.

ancestral right hypobranchial gland (Fretter et al., 1998), it appears that the capacity for Tyrian purple synthesis has been retained in various reproductive glands of the Muricidae over the course of evolution. The presence of Tyrian purple precursors (Benkendorff et al., 2001, 2004) in the egg masses of species from the monophyletic subfamilies Rapaninae, Muricinae, Ocenebrinae, and Ergalataxinae (Claremont et al. 2008) suggests this phenomenon is widespread in the Muricidae. However, the absence of Tyrian purple precursors from some Ocenebrinae species (Benkendorff et al., 2001, 2004) indicates that gonoduct biosynthesis of hypobranchial gland metabolites may be clade-specific. Nevertheless, this chemotaxonomic divide coupled with male Tyrian purple genesis (Elsner and Spanier, 1985; Verhecken, 1989; Michel et al., 1992; Benkendorff et al., 2004; Westley and Benkendorff, 2008), indicates that maternal provisioning to support larval development is not the sole function of these natural products.

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Trends in molluscan gene sequence similarity: An observation from genes expressed within the hypobranchial gland of *Dicathais orbita* (Gmelin, 1791) (Neogastropoda: Muricidae)

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ABSTRACT

This study investigates the phylogenetie distribution of homology to *Dicathais orbita* hypobranchial gland genes based on tBLASTx pairwise sequenee alignments from the Genbank database. Suppressive subtraetive hybridization was used to obtain 417 non-redundant genes that were up-regulated or uniquely expressed in the hypobranchial gland relative to mantle tissue. Of these, 133 sequenees revealed matehes to the database with the remaining 68% of genes appearing as apparently novel sequences. Homologous sequenee matches were observed for a wide range of evolutionarily divergent taxa, eneompassing animals, protozoans, plants, fungi, bacteria, and viruses. The highest frequency of homology was found towards ehordate sequenees, followed by the Mollusea, whieh highlights the current bias in availability of vertebrate versus invertebrate sequences in the database. An unexpectedly high proportion of matches were also found toward the Ciliophora, indieating a possible symbiotie relationship, as well as the Ascomycota and Streptophyta, which share the ability to biosynthesize indole derivatives with Muricidae such as *Dicathais orbita*. Overall, these results reveal the usefulness of undertaking sequence comparisons in gene expression and highlight the current paucity of knowledge of molluscan genomes.

Additional keywords: Gastropoda, DNA

INTRODUCTION

The development of genomic technologies has had a dramatic effect on all fields of biological sciences (Collins et al., 2003). Since the completion of the human genome project in 2003 (Collins et al. 2003), the number of genomes available has grown dramatieally. As of November 2007, a total of 426 eukaryotic (24 complete, 164 undergoing assembly and 238 in progress) and 599 bacterial genomes were available on the Genbank database (NCBI, 2007). The inereased number of genomes available enhanees our understanding of the biology of the species in question and provides a basis for eomparative studies in functional biology. Despite this increase in data, trends in eomparative genomies favor the analysis of mammalian sequences (Barnes et al. 2004), and often the homologous identifieation and classification of non-vertebrate sequenees is more challenging.

The Mollusea has been identified as the second most diverse and speciose phylum in the animal kingdom, with members present in marine, freshwater, and terrestrial environments (Peehenik, 2000). Despite their abundance and the eeonomie importance of many species (Beesley et al., 1998), the genome of mollusks remains relatively uneharted. So far, the eomplete genome has only been sequenced for the Californian sea hare *Aplysia californica* Cooper, 1863, and this is yet to be annotated (NCBI, 2007). The bivalves *Argopecten irradians* (Lamarck, 1819), *Crassostrea virginiea* (Gmelin, 1791), and *Spisula solidissima* (Dillwyn, 1817), which are all important fisheries resources, and the medieally important freshwater snail *Biomphalaria glabrata,* are eurrently undergoing sequencing (NCBI, 2007). Nevertheless, a major hurdle in molluscan genomies lies in defining the funetions of sequenees identified. Sequence homology has been used heavily to assign functions in mammalian genomes. However, the lack of currently available molluscan and invertebrate sequenees limits the ability to assign gene funetion using comparative elassifieations drawn from existing invertebrate genomic sequence information. Nevertheless, broader eomparisons to more distantly related organisms could yield novel information about well conserved genes or genes that have independently evolved convergent functions in distinct taxa.

The hypobranehial gland of neogastropods is a uniquely molluscan organ (Beesley et al., 1998) of uncertain

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origin and function (Westley et al., 2006). Within the family Muricidae, it is the well known source of the ancient dye Tyrian purple (Baker, 1974; Cooksey, 2001). Tyrian purple is generated by a series of chemical reactions from indoxyl sulphate precursors that are brominated secondary metabolites thought to be derived from the amino acid tryptophan (Westley et al., 2006). While the Muricidae are thought to be the only source of the purple brominated dye, the related blue dye indigo is produced by a number of other taxa including plants, bacteria and fungi (Epstein et al., 1969; Meijer et al., 2006; Mayser et al., 2007). This presents an interesting case of apparent convergent evolution in biosynthetic capabilities.

Basic Local Alignment Search Tool (BLAST) analysis is a key tool used to identify orthologous genes from different organisms, and its use has been instrumental in classifying countless sequences (Galagan et al., 2003; Venter et al., 2001). The taxonomic classifications of high scoring BLAST matches with unclassified sequences are useful in identifying sequences with specific or variable functions and may indicate key gaps in the current sequence data for members of specific phyla. This study results from a larger project that is currently underway to identify the genes expressed in the hypobranchial gland of *Dicathais orbita* (Gmelin, 1791), a predatory marine gastropod belonging to the family Muricidae, order Neogastropoda. Here we report on our tBLASTx analysis, where sequences were translated into all possible protein translations and compared to all possible translations of every nucleotides sequence in Genbank, to observe trends in molluscan sequence similarity and assess the proportion of homologous genes expressed in this unique biosynthetic organ.

MATERIALS AND METHODS

A suppressive subtractive hybridization (SSH) (Diatchenko et al., 1999) cDNA library containing the up-regulated and differentially expressed genes within the hypobranchial gland of *D. orbita*, when compared to mantle tissue gene expression, was created using a Clontech PCR-Select™ cDNA Subtraction Kit (Clontech, California, USA). The RNaqueous® RNA extraction kit (Ambion, Texas, USA), TRI Reagent® (Ambion) and DNaseI (Invitrogen, CA, USA) digestion were used to obtain RNA from the hypobranchial glands and mantle of two *D. orbita* specimens. The subtraction was performed utilizing pooled hypobranchial gland transcripts as the tester population and pooled mantle transcripts as the driver population. Subtracted cDNA produced from SSH were cloned into pGEM®-T Easy vector (Promega, Wisconsin, USA). Colonies with inserts were selected, plasmid DNA was purified and sequencing was performed by Southpath and Flinders Sequencing Facility (Adelaide, Australia) or Australian Genome Research Facility (AGRF sequencing, Brisbane, Australia). A total of 554 plasmids were sequenced, and vector sequence and adaptor regions were removed. Contigs were formed using Sequencher Version 4.1.4 yielding a non-redundant set of expressed sequence tags (EST's) differentially expressed in the hypobranchial gland of *D. orbita*. In total, 417 unique resulting sequences were submitted to tBLASTx analysis and the highest scoring matches for all sequences with an e value smaller than $1e^{-5}$ were collated. The phylum of the orthologous sequence was recorded, and in cases where the highest scoring tBLASTx matched a molluscan sequence, the class was determined. In cases where the matching sequence belonged to a member of the class Gastropoda, the family was also recorded. The total number of Genbank sequences for phyla with 5 or more sequence matches was recorded (Figure 1).

RESULTS

A total of 133 sequences out of 417 (31.9%) resulted in significant tBLASTx matches, with 23 different phyla represented from the best scoring blast match for each identified sequence. Seven of these phyla had matches to 5 or more hypobranchial gland sequences from *D. orbita*. The Chordata showed the highest number of matches, with 32 homologous sequences identified, closely followed by the Mollusca with 31 matches (Figure 1). Ciliophora were the third most abundant phylum with 15 matches, followed by the invertebrate phyla Arthropoda and Echinodermata, with 12 and 8 sequences identified, respectively (Figure 1). There were seven Ascomycota homologs identified in *D. orbita's* hypobranchial gland, as well as five from the Streptophyta (Figure 1).

Of the 31 molluscan sequence matches identified, 22 sequences matched gastropod sequences. Twelve of these gastropod sequence homologs belonged to other members of the Muricidae family (Figure 1). Further distribution of the sequence homology is detailed in Figure 1.

DISCUSSION

While 133 of the sequences produced had BLAST matches that indicated the function of the transcripts, the remaining 284 genes sequenced from the hypobranchial gland of *Dicathais orbita* appear to be novel, highlighting the limited information currently available on molluscan genomes. The high frequency of matches to chordate sequences is likely to be due to the large abundance of vertebrate sequences in the public database (Barnes et al., 2004) (Table 1).There are currently over 57 million gene sequences from the Chordata, compared to less than 600,000 molluscan sequences available (Table 1). There is clearly a bias towards a high proportion of tBLASTx matches returning matches to human and other chordate sequences, which have over 90 times the number of molluscan genes available for sequence alignment.

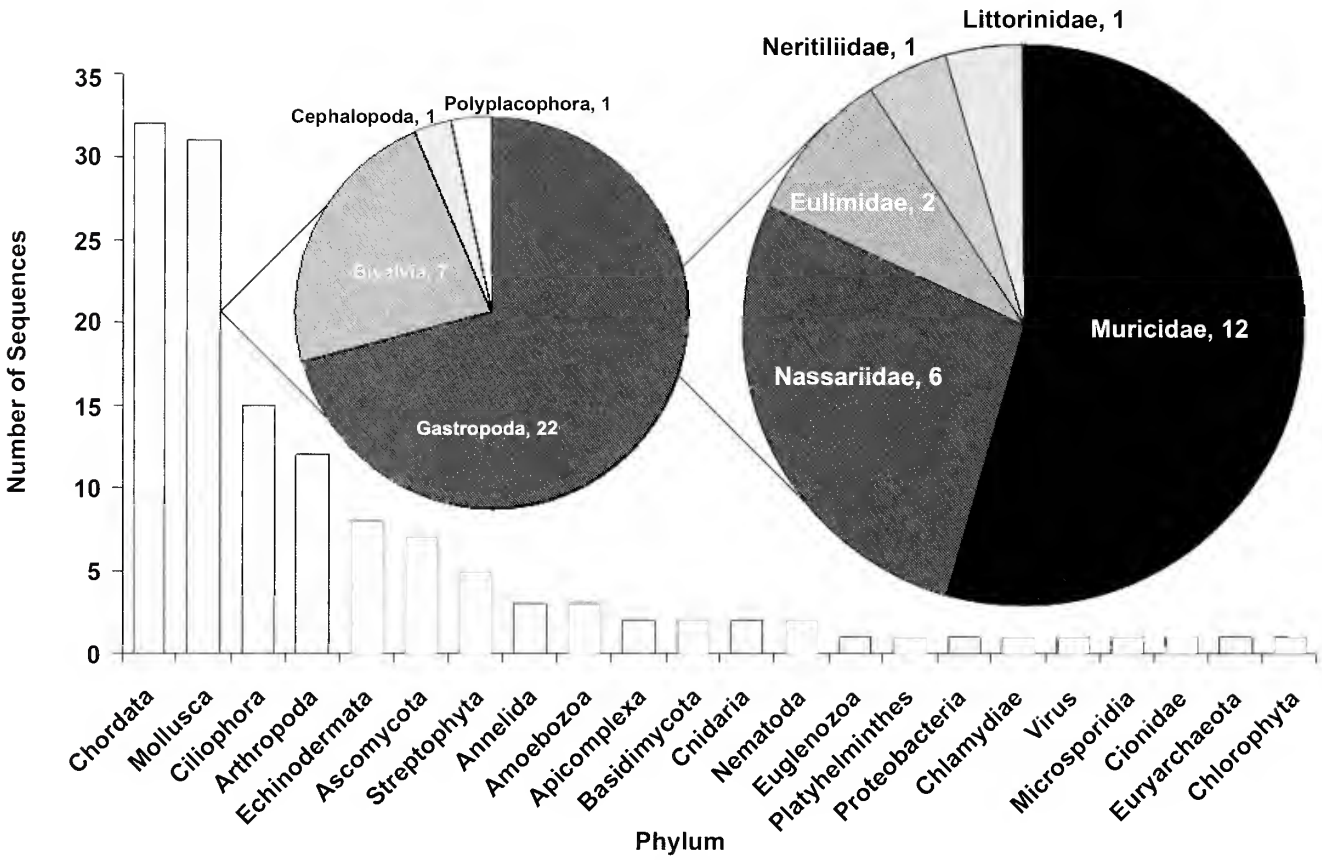

Figure 1. Phyla represented by highest scoring tBLASTx matches of genes expressed in the hypobranchial gland of *Dicathais orbita*. A total of 417 non-redundant EST sequences were analysed using tBLASTx and the resulting 133 significant matches (E value $< 10^{-5}$) were placed into 23 categories, based on the phylum grouping of the highest-scoring tBLASTx matches. Sequences grouped in the phylum Mollusca were further classified into the corresponding Class of the best tBLASTx match. Gastropod sequences were further divided according to family of the highest scoring tBLASTx matches.

The abundance of matches to sequences from the Ciliophora was unexpected, particularly since the number of ciliate sequences in public databases is just over 300,000 (Table 1). It is possible these protozoan gene matches actually result from ciliate genomes derived from endosymbionts occurring within the hypobranchial gland of *D. orbita*. Ciliates are ubiquitous protists that commonly form relationships with other species, such as the parasitic *Ichthyopthirius multifilius* (Abernathy et al., 2007) and the symbiotic *Euplotes uncinatus* (Lobban et al., 2005).

The abundance of matches to arthropod species was not unexpected due to the shared ancestral relationship between the Mollusca and Arthropoda. However, the numerous matches to Echinodermata are less expected given that this phyla occurs on the deuterostome lineage along with chordates, which diverged from the mollusks and other protostomes over 100 million years ago (Heckman et al., 2001). Notably, there were relatively few matches to the Annelida (Figure 1) despite the fact that this abundant protostome phylum occurs within the Lophotrochozoan lineage alongside the Mollusca, which form a separate clade from the Ecdyzoa, including arthropods and nematodes (Aguinaldo et al., 1997). It is likely that the small number of annelid sequences available, less than 35, 000 (Table 1), contributed to the small incidence of annelid sequence homology with our molluscan sequences. This further highlights the relatively limited genetic information that is available for so called "primitive" invertebrate phyla.

Table 1. Number of nucleotide sequences available on Genbank database for different phyla as published on the 18 December 2007. All data was compiled as published under the Taxonomy browser available on NCBI Entrez taxonomy home page http://www.ncbi.nlm.nih.gov/sites/entrez?db=Taxonomy.

Phylum	Genbank nucleotide sequences
Chordata	57,495,211
Mollusca	599,894
Ciliophora	303,304
Arthropoda	5,090,469
Echinodermata	941,561
Ascomycota	1,367,029
Streptophyta	22,413,399
Annelida	34,245

The frequency of sequence matches to the fungal Ascomycota and the plant Streptophyta was an unexpected finding. This is possibly related to the fact that members of both the Streptophyta and Ascomycota are capable of similar secondary metabolite production as is the muricid *Dicathais orbita*. Indigo is produced in *Isatis tinctoria* (phylum Streptophyta) (Epstein et al., 1967), and the production of indole compounds has been reported for *Candida glabrata* (phylum Ascomycota) (Mayser et al., 2007). These compounds are in the same chemical class of indole alkaloids as Tyrian purple, the brominated derivative of indigo secreted only from the hypobranchial gland of the Muricidae (Cooksey, 2001; Westley et al., 2006). These similarities in secondary metabolite production may influence the frequency of homology with genes expressed in the hypobranchial gland of *D. orbita*. Further analysis of the conserved genes could help reveal some key biosynthetic enzymes and/or processes. As SSH allows for amplification of only up-regulated or uniquely expressed genes in this instance, we would expect sequences involved in chemical and protein biosynthesis to be amplified. This demonstrates that it is important to consider the source of expressed genes when interpreting sequence homology.

Another key observation is the frequency and variation of molluscan gene matches observed from our tBLASTx analysis. As mentioned, a total of 31 molluscan sequence matches were identified, with 22 gastropod sequences, 12 of which belonged to the family Muricidae (Figure 1). This trend is expected as species within the same family are expected to show greater homology with our *D. orbita* sequences. The key limiting factor to the number of muricid and gastropod sequence matches is the limited amount of sequencing that has been performed on these groups, only 1994 Muricidae sequences have been published on the NCBI database as of November 2007 (NCBI 2007). The majority of sequences available for muricids are highly conserved genes involved in phylogenetic analysis such as ribosomal RNA (Colgan et al., 2007; Harasewych et al., 1997; Oliverio and Mariottini, 2001), cytochrome oxidase 1 (Colgan et al. 2007; Harasewych, et al., 1997) and histone H3 sequences (Colgan et al., 2007). The frequency of positive matches to *D. orbita* hypobranchial gland genes is likely to increase as a broader range of sequences from additional Muricidae and other gastropod species are made available on Genbank.

From tBLASTx analysis, we have identified the phylogenetic distribution of species that share homology with *Dicathais orbita* gene sequences. While less than 32% of sequences could be positively matched on the gene databases, 31 matches were found encompassing species from both invertebrates and vertebrates within the Animal Kingdom, as well as eukaryotic plants, protozoans, fungi, some prokaryotes and even viruses. Most matches pertain to chordate sequences, and this may be attributed to the abundance of these sequences within databases. Nevertheless, many of the sequences match other molluscan species and other invertebrate phyla, likely due the close evolutionary relationships leading to conserved genes. A significant proportion of sequences belong to ciliate protozoans, and it is unclear whether this is due to similarities between these protists and *D. orbita* or the addition of ciliate genes within our hypobranchial gland expressed genes. The limited number of molluscan gene matches from our dataset supports the need for a larger number of molluscan sequences to be identified and released, encompassing a broader range of functional genes. Only then will we be able to accurately view trends in gene expression within the hypobranchial gland of *D. orbita*.

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Feeding behavior of *Adelomelon ancilla* (Lighfoot, 1786): A predatory neogastropod (Gastropoda: Volutidae) in Patagonian benthic communities

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ABSTRACT

Adelomelon ancilla, a volutid commonly found in shallow water in northern Patagonia, is a top predator in the benthic communities of this region. This species presents an anemone (*Antholoba achates*) epibiosis that may protect it from predators. *Adelomelon ancilla* captures prey by tightly engulfing it with the foot, and ingests them, generally alive, after narcotizing their muscles. A narcotizing substance, produced by the accessory salivary glands, is released through the proboscis into the prey while the latter is tightly enveloped within the foot, allowing for prey narcotization. In this space, water is not abundant and, therefore, the salivary secretion reaches a high concentration, with a pH of around 10. Analysis of prey obtained *in situ* indicated that *A. ancilla* mainly consumes bivalves (88.9%), gastropods (9.5%) and, rarely, sea urchins (1.6%). Ingestion of the prey usually occurs while the predator is buried in the substrate, and may last for several hours. The anatomy of the alimentary system and the pH of various organs involved in prey capture and digestion are presented along with a comparison with feeding mechanisms among other species of Volutidae.

Additional keywords: Neogastropoda, feeding mechanism, saliva, Patagonian benthos

INTRODUCTION

Adelomelon ancilla (Lightfoot, 1786) is a neogatropod belonging to the family Volutidae, subfamily Zidoninae. It occurs along the western Atlantic coast of South America from 35° S southward to Ushuaia Bay, the Beagle Channel (G. Bigatti, pers. observ.), through the Straits of Magellan, and northward into the Pacific, reaching Chiloé Island in Chile (Castellanos and Landoni, 1992). In the gulfs of northern Patagonia, this species inhabits mixed gravel and sand bottoms, and is easily collected by SCUBA at depths of 5 to 20 m, during low tide, and near the shore. Despite its commercial importance as a new fishery resource, *A. ancilla* has not been well studied, with research on this species being limited to descriptions of egg capsules and embryology (Penchaszadeh and De Mahieu, 1976; Penchaszadeh et al., 1999; Penchaszadeh and Miloslavich, 2001; Penchaszadeh et al., 2006), and to reproductive biology and oviposition (Penchaszadeh et al., 2006; Penchaszadeh et al., 2009). Bigatti and Ciocco (2008) pointed out that this species constitutes a new fishery resource for artisanal fishing communities in northern Patagonia, but fishing policies for the species have not yet been established.

Taylor et al. (1980) noted that neogastropods comprise the majority of predatory gastropods, which are important and abundant components of shallow water communities. The act of predation comprises a series of complex behaviors including search, capture, immobilization, penetration of prey and, finally, ingestion. Predators differ from other gastropods in their anatomical and behavioral features. Ponder (1974) reported on anatomical features that differentiate neogastropods from other higher Caenogastropoda. Many of the derived features are in the anterior alimentary system, and include the formation of an eversible proboscis, a modified radula, a valve of Leiblein, and generally two pairs of salivary glands. Others features include a well developed siphon and a complex osphradium, both for improved chemoreception. Indeed, most families of Neogastropoda are differentiated based on anatomical differences related to feeding.

Feeding mechanisms have not been studied for most species of Volutidae. Bigatti (2005) reported that the

Patagonian volutid *Odontocymbiola magellanica* (Gmelin, 1791), which occurs sympatrically with *A. ancilla*, engulfs its prey with its foot, creating a chamber into which it releases saliva in order to narcotize the prey. Weaver and Dupont (1970) reported that *Alcithoe arabica* preyed on bivalves and other gastropods as suggested by other authors for other members of the Volutidae (Taylor et al., 1980; Ponder, 1970).

In this paper we describe the feeding mechanism, prey preferences, anemone epibiosis, anatomical features of the alimentary system of *Adelomelon ancilla*, and compare it with the information available for other volutids.

MATERIALS AND METHODS

Study Area and Habitat: The sediments at Golfo Nuevo, Argentina are mixed, being composed of sand, mud, and/or gravel. Mollusks occur in low densities. The bivalves prevalent in the study area are *Aulacomya atra* (Molina, 1782), *Protothaca antiqua* (King and Broderip, 1832), and *Eurhomalea exalbida* (Dyllwin, 1817), and tend to occur in patches. The scallop *Acquipecten tehuelchus* (d'Orbigny, 1842) is also present, but is very widely distributed. The algal assemblage is dominated by *Codium vermilara* (Olivi) and *Dictyota dichotoma* (Hudson), in addition to other small algal species, and hosts populations of the gastropods *Buccinanops globulosus* (Kiener, 1834), *Notocochlis isabelleana* (d'Orbigny, 1840), and *Tegula patagonica* (d'Orbigny, 1840).

Sampling: Sampling was performed by SCUBA diving in Golfo Nuevo, Patagonia Argentina (42°46' S, 64°59' W) at 5–20 m depths depending on the tide. Predator and prey were collected together and processed in the laboratory. The lengths of predator and prey were measured, and the correlation between prey and predator size analyzed.

The number of anemones on the snail's shell, and the fraction of the shell surface covered by anemones was calculated, allowing for an estimate of shell surface area as length × width, and the anemone surface area as πr^2 (with r = average of major and minor radius of anemone).

Anatomy and pH of Alimentary System: The alimentary systems of feeding and non-feeding animals were dissected. Salivary glands (**SG**), accessory salivary glands (**ASG**), glands of Leiblein and stomachs were separated, and their pH determined for 39 individuals. Each freshly dissected organ was diced using dissecting scissors, placed in a vial with distilled water and stirred using a magnetic stir bar. The pH was measured using a digital pH meter (MV-RS 232; 0.01 unit) or pH indicator paper (Merck, range 0–14).

Feeding Mechanism and Prey Items: *Adelomelon ancilla* were observed while capturing prey and photographed *in situ* to record the feeding mechanism and time of ingestion. Upon return to the laboratory, the predators' stomachs were dissected and their contents examined under a stereoscopic microscope to identify the ingested prey remains.

RESULTS

Adelomelon ancilla are normally infaunal (Figure 1), and may be detected from above by the small mound of sediment they make on the bottom, with the apex or the siphon exposed, or because they carry the sea anemone *Antholoba achates* (Drayton *in* Dana, 1846) as an epibiont.

This anemone was present on 98% of the *Adelomelon ancilla* sampled (n=39 snails; Figure 2), with 1–6 anemones attached to the dorsal shell surface of each individual (mean= 2.00; SD= 1.26). The surface area of the snails occupied by the anemones ranged from 1.6% to 98.0% (mean= 34.2; SD= 29.5). In addition to the symbiosis with an epibiotic anemone, another distinctive external character of *A. ancilla* that differentiates it from the sympatric volutid *Odontocymbila magellanica* is the violet to pale violet color of its foot (red in *O. magellanica*) and the more elongated shell shape (Figures 3, 4).

Anatomy and pH of the Alimentary System: The anterior portion of the alimentary system of *Adelomelon ancilla* (Figure 5) contains a pleurembolic proboscis and paired white accessory salivary glands (**ASG**) that are "loosely wound" around brown (light brown to reddish brown) salivary glands (**SG**), as illustrated by Clench and Turner, (1964: pl. 82, fig. 26). The secretion of the **ASG** is a white and viscous fluid, similar to that released at the distal end of the proboscis when the snails are disturbed. Both **ASG** and **SG** are situated anterior to the valve of Leiblein (Figure 6). Ducts of the **ASG** and **SG** are very thin and run parallel to the anterior esophagus. The **ASG** ducts join at the tip of the proboscis, while the **SG** ducts become embedded in the anterior esophagus at mid-length and enter the buccal mass. The valve of Leiblein, situated posterior to the salivary glands and anterior to the nerve ring (Figure 6), separates the anterior esophagus from the mid-esophagus. The gland of Leiblein (Figure 5), which is relatively long and surrounded by connective tissues, joins the mid-esophagus posterior to the valve of Leiblein. The posterior esophagus leads from the mid-esophagus to the U-shaped stomach, which is embedded in the digestive gland. Posterior to the stomach is the rectum and then the anus which presents a pyramidal papilla.

The pH of macerated fresh organs (and their secretions) from the alimentary systems of 39 animals of *A. ancilla* are reported in Table 2. As a general rule, the pH in the alimentary system anterior to the valve of Leiblein was alkaline (pH≈10), while posterior to the valve of Leiblein, the pH was nearly neutral (pH≈7).

Feeding Behavior: Observations in the field revealed that individuals of *Adelomelon ancilla* capture their prey by enveloping them with the foot (Figures 3 and 4), creating a chamber that is closed but not totally isolated from the environment. After some hours, the prey is narcotized by a secretion (pH≈10) produced by the accessory salivary glands and released into this chamber from the proboscis. As there is little water in this cham-

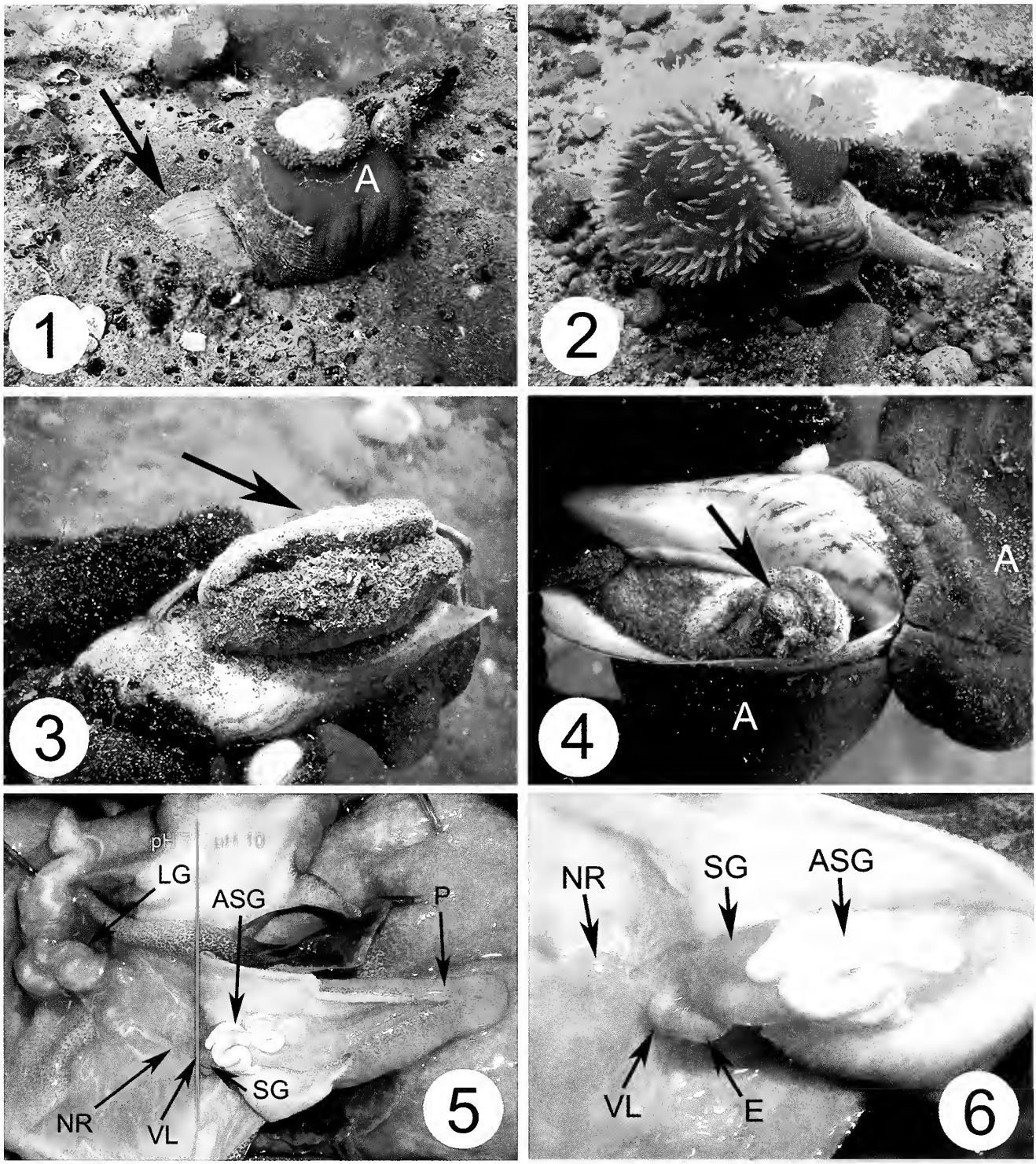

Figure 1–6: *Adelomelon ancilla*. **1–4.** In its natural environment (mixed bottoms of gravel and sand) at Golfo Nuevo, Patagonia, around 100 mm shell length. **1.** Individual of *A. ancilla* buried in the substratum as commonly found. Arrow shows the shell. **2.** An individual with 2 anemones *Antholoba acathes* fixed in the shell. **3.** Specimen engulfing a prey by the foot (arrow). **4.** Same individual showing the prey, *Tegula patagonica* (arrow). **5–6.** Anatomy of the anterior digestive system of *Adelomelon ancilla*. **5.** General view of the anterior digestive. **6.** Detail of salivary glands. Abbreviations: **A,** anemone; **ASG,** accessory salivary gland; **E,** esophagus; **LG,** Leiblein gland; **NR,** nerve ring; **P,** eversible proboscis; **SG,** salivary gland; **VL,** valve of Leiblein.

ber, the accessory salivary gland secretion reaches a high concentration. The effect of the narcotizing substance is to produce muscular relaxation in the prey: bivalves open the valves by releasing their adductor muscles, while gastropods lose the ability to contract their columellar muscles. A second effect appears to be a decreased speed of muscle reaction/contraction, enabling the predator to use its radula to feed on living prey tissues. Most of the individuals of *A. ancilla* that were observed feeding were buried in the substrate.

PREY: A total of 63 individual prey were sampled from feeding *A. ancilla*. Prey consisted mainly of bivalves, with a smaller proportion of gastropods, and rarely sea urchins (Figure 7). The bivalves eaten were *Protothaca antiqua, Eurhomalea exalbida, Aulacomya atra,* and *Diplodonta patagonica*. Gastropod prey consisted of *Tegula patagonica, Notocochlis isabelcana,* and *Crepidula dilatata*. The green sea urchin *Arbacia dufresnii* was eaten in less than 2 % of the studied cases (Table 1).

There was no significant correlation between predator size and prey size (R^2=0.0092) (Figure 8): we observed large predators ingesting small prey as well as small predators ingesting large prey. No cases of cannibalism were observed in this study. From all the stomach contents analyzed (n=39), only two contained the remains of the ambulacral system of an unidentified small sea star; the rest contained a light brownish mucous or were empty.

DISCUSSION

As noted by Leal and Bouchet (1989: 11), *Adelomelon ancilla* has been commonly confused with the sympatric *Odontocymbiola magellanica* due to convergence in external shell morphology. These species differ in foot coloration (violet in *A. ancilla* and intense red in *O. magellanica*), and *A. ancilla* has a more elongated shell with sea anemones attached to the dorsal part of the shell in the study area. These characters serve as convenient means of differentiating these volutes in the region of Golfo Nuevo, Argentina. Significant differences in the shape of the rachidian tooth and in the morphology of the salivary and accessory salivary glands easily allow for the correct identification of both species to their respective subfamilies, *Adelomelon ancilla* to Zidoninae and *Odontocymbiola magellanica* to Odontocymbiolinae (Clench and Turner, 1964).

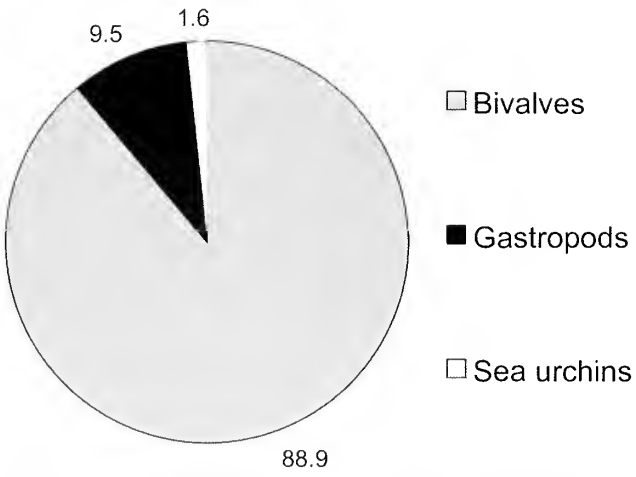

Figure 7. Proportion of prey taxa eaten by *Adelomelon ancilla*, expressed as percent, based on 63 observations.

Table 1. Prey consumed by *Adelomelon ancilla* in Golfo Nuevo, Argentina.

Prey	N	%
Protothaca antiqua	35	55.6
Eurhomalea exalbida	17	27.0
Tegula patagonica	4	6.3
Arbacia dufresnii	1	1.6
Aulacomya atra	3	4.8
Notocochlis isabeleana	1	1.6
Diplodonta Patagonica	1	1.6
Crepidula dilatata	1	1.6

ANATOMY AND PH OF ALIMENTARY SYSTEM: The anatomy of the alimentary system of *A. ancilla* agrees with published reports for the family Zidoninae (e.g., Clench and Turner, 1964: pl. 82, fig. 26; Leal and Bouchet, 1989: fig. 32). The valve of Leiblein does not allow for the reflux of the secretions from the middle esophagus or the gland of Leiblein into the anterior esophagus (Ponder, 1974; Andrews and Thorogood, 2005; Kantor and Fedosov, 2009). The pH of the anterior alimentary system is around 10 (Table 1, Figure 7), while the pH of the middle and posterior alimentary system is around 7. The same conditions were observed in *Odontocymbiola magellanica* by Bigatti (2005), in a study of the diet, feeding behavior, and biochemical composition of the saliva of this species. Bigatti (2005) hypothesized that "the ducts of the salivary gland which finish in the anterior esophagi and pour their secretion in that area, would avoid the contact of the narcotizing liquid ingested (from ASG) together with the prey, covering the esophagic [sic] epithelium with saliva (without the narcotizing compound), prior to the release of the accessory salivary glands secretion. After passing through the Leiblein valve, the pH of the digestive system decreased up to approximately 7.5. This shift in pH would allow the inactivation of the salivary fluid carrying the narcotizing function, avoiding toxicity for the producer in the digestive system." The same processes to prevent the

Table 2. pH from freshly dissected digestive organs of *Adelomelon ancilla*. Abbreviations: ASG: accessory salivary gland; SG: salivary gland; LEIBLEIN: gland of Leiblein.

	ASG+SG	ASG	SG	LEIBLEIN	STOMACH
Mean	10.06	9.93	9.69	6.94	7.06
SD	0.91	0.30	0.85	0.35	0.17

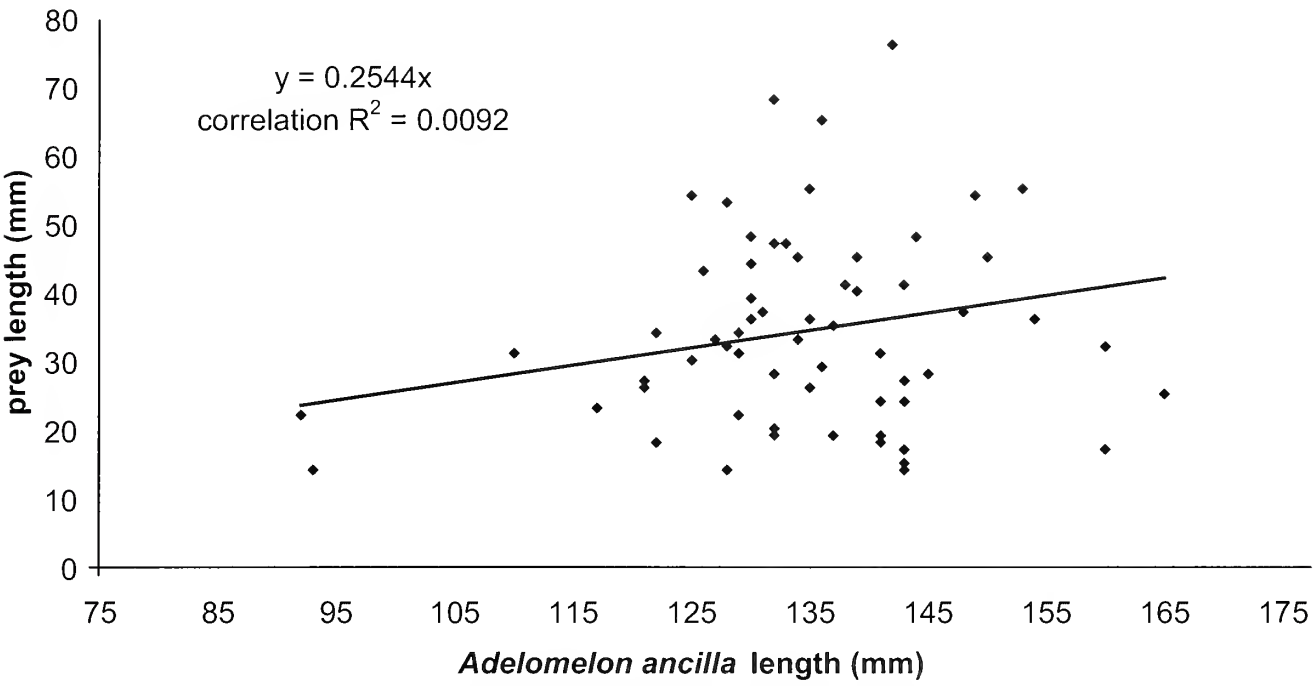

Figure 8. Correlation between predator and prey sizes. No significant correlation was found.

secretion from the accessory salivary gland from affecting the foregut of the predator may occur in *A. aneilla*, but we did not perform specific studies to assess this. Andrews (1991) stated that gastropod salivary gland secretions have different physiological functions, including lubrication and food ingestion, as well as the initial phase of external digestion and prey capture. *Cymatium intermedium* (Pease, 1869) has six types of salivary secretions with activities that include enzymatic, toxic, acidic and protection of the digestive tract (Andrews et al., 1999). In this work we only analyzed the pH of different fresh organs of the alimentary system. A more detailed study of the biochemistry of salivary gland and accessory gland secretions is clearly needed to clarify the physiology of the feeding mechanism in *Adelomelon ancilla*.

Feeding Behavior: Feeding mechanisms have been described for relatively few species of Volutidae. Morton (1986) reported that *Melo melo* (Lightfoot, 1786) covers the prey (mainly gastropods) with its foot, forming a sealed chamber, possibly secreting a toxin by means of the salivary glands to kill the prey. Novelli and Novelli (1982) reported that the volutid *Adelomelon brasiliana* (Lamarck, 1811) (from southern Brazil) also covers prey with its foot, and suggested that prey are killed by asphyxia. These authors observed a white viscous fluid coming from the mouth, and believed it to be a narcotizing compound. Taylor et al. (1980) and Ponder (1970) suggested that volutids asphyxiate their prey by enveloping them with the posterior part of the foot.

Our results are similar to and suggest the same feeding mechanisms as those observed for *Odontoeymbiola magellanica* (Bigatti, 2005). Prey are probably narcotized by the secretion produced by the accessory salivary glands and applied through a duct opening at the ventral tip of the mouth, then ingested alive. Although the time of ingestion was not established for *A. aneilla* (because it is longer than the time a diver can remain underwater), our hypothesis is that it could be similar to that for *O. magellaniea*, or approximately ten hours (Bigatti, 2005). This slow pace of feeding may be related to the temperate environment (8–18°C) inhabited by the snails; for the tropical volutid *Voluta ebraea*, the total consumption of a prey takes 40 minutes (Bigatti and Matthews-Cascon, pers. observ.).

Prey: The analysis of prey obtained *in situ* indicated that *A. aneilla* consumes mainly bivalves (88.9%) and gastropods (9.5%), with a single report of a sea urchin (1.6%). Studies of relative abundances of the benthic species were not conducted. *Adelomelon aneilla* was found primarily associated with patches of bivalves, in soft and mixed bottoms, rather than in rocky or hard bottoms. Other snails inhabiting soft bottoms in the area were the volutid *O. magellanica* (which was not found to be either prey or predator) and the naticid *Natica isabelleana* (ingestion=1.6%). However, the hard bottom gastropod species *Tegula patagonica* and *Crepidula dilatata* were infrequently preyed upon (6.3% and 1.6% respectively), suggesting forays by *A. ancilla* onto hard substrates.

Other reports of volutid prey include those of Weaver and Dupont (1970), who noted that the related congener *Adelomelon beckii* (Broderip, 1836) (also from Argentinean waters) "is captured by means of hooks

with bait, raising the assumption that this species is carnivore". Studies of stomach content in *A. beckii* from Mar del Plata and Quequén coasts revealed the presence of muscle tissues of another volutid, *Zidona dufresnei* (Donovan, 1823) (Floreneia Arrighietti, pers. comm.). The volutid *Melo amphora* (Lightfoot, 1786) was studied by Wilson and Gillet (1971) who showed a specimen feeding on another volutid, *Zebramoria zebra* (Leach, 1814). The absence of volutids captured in baited traps of the local snail fisheries (Bigatti and Cioeco, 2008) suggests that the species from Patagonian waters are predators rather than carrion feeders. The starfish remains found in the stomachs as well as the direct observation of predation on sea urchins, reinforces the fact that *A. ancilla* does not feed exclusively on mollusks as do *O. magellanica* and the other volutids studied to date. Taylor et al. (1980) noted that members of the Volutidae are mainly predators on bivalves and gastropods. While neither cannibalism nor predation on other volutids was recorded for *A. ancilla*, it was observed at a low rate (4.7%) in *O. magellanica* (Bigatti, 2005). The differing proportions of prey organisms captured by *A. ancilla* (bivalves, 88.9%; gastropods, 9.5%), and *O. magellanica* (bivalves, 46%; gastropods, 54%) may be indicative of slight niche partitioning among these sympatric species at Golfo Nuevo.

The results presented in this paper are a first approach to the study of the feeding behavior of *Adelomelon ancilla*, and help to understand the relationship with its sympatric species *O. magellanica*. The sea anemone *Antholoba achates* (Drayton in Dana, 1846) is very unusual as an epibiont of *O. magellanica* (observed in less than 1% of snails, Bigatti, pers. observ.). Both snails are top predators in the benthic communities they inhabit, but *O. magellanica* is preyed upon (at low rates) by local fishes (Galvan, 2008), while *A. ancilla* is not, likely due to the protection provided by the epibiont. The same species of anemone was observed as an epibiont on *Adelomelon brasiliana*, another volutid from the northern coasts of Argentina (Luzzatto and Pastorino, 2006). These authors believed that the anemone does not provide any benefit for the snail, but rather, hinders its normal motion. The relationship between *Adelomelon ancilla* and *Antholoba achates* has not yet been studied, leaving unanswered, the question of why 98% of the specimens of *A. ancilla* have at least one epibiont anemone while the co-occurring *O. magellanica* has none.

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Sperm morphology of two marine neogastropods from the southwestern Atlantic Ocean (Caenogastropoda: Volutidae and Olividae)

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ABSTRACT

The testes of Caenogastropoda typically produce two types of spermatozoa, euspermatozoa and paraspermatozoa. The structures of both morphological forms of sperm contribute to our understanding of reproductive biology, and also have been useful to elucidate taxonomic and phylogenetic relationships among gastropods. This article describes the ultrastructure and the possible importance for systematics of the euspermatozoa in two species, *Adelomelon beckii*, family Volutidae, and *Olivancillaria deshayesiana*, family Olividae.

The euspermatozoa of these species are characterized by: the presence of an acrosomal vesicle with an apical bleb and accessory membrane; a nucleus that is long and tubular with the axoneme penetrating the nucleus; a midpiece with mitochondrial elements coiled helically around the axoneme; a glycogen piece; and a short end piece. A constriction in the acrosomal vesicle and mitochondrial elements that appear U-shaped and electron dense in cross section are features that are present in the studied taxa, but have not been reported outside of the Neogastropoda.

Additional keywords: Sperm, ultrastructure, Gastropoda, Neogastropoda

INTRODUCTION

Members of the family Volutidae are active marine predators. The majority of taxa inhabit sandy to silty bottoms in coastal waters of the southern hemisphere, although the family has a global distribution and extends to bathyal and abyssal depths (Clench and Turner, 1970; Poppe and Goto, 1992). More than 200 species are known, with shells that vary substantially in shape and size. Fourteen species of Volutidae are reported from the southwestern Atlantic Ocean, including members of the genus *Adelomelon* (Rios, 1994). *Adelomelon beckii* (Broderip, 1836) is endemic to the southwestern Atlantic Ocean, ranging from Espírito Santo, Brazil, to Tierra del Fuego, Argentina. It is the largest (390 mm maximum length) carnivorous gastropod in the region, and inhabits sandy bottoms at depths of 35 to 70 m (Poppe and Goto, 1992). *Adelomelon beckii* has been caught as a byproduct of trawler fishing, but in the past several years a new market demand appeared for this species. Its large, muscular foot is sold for food, while its shell is sold in local markets as an artisanal product.

The family Olividae encompasses carnivorous, infaunal marine gastropods of medium size (Smith, 1998). Olivids inhabit nearshore waters along the northern coast of Argentina. Twelve species of Olividae are reported off the Argentine coast, spanning the genera *Olivancillaria*, *Olivella*, and *Amalda*. Seven species of *Olivancillaria* are recorded from South America (Castellanos, 1970; Rios, 1994).

Olivancillaria deshayesiana (Ducros de Saint Germain, 1857), with a maximum shell length of 35 mm, is the most common species. It is distributed along the southern coast of Buenos Aires province and lives at depths of 6–12 m, from Rio de Janeiro to Mar del Plata. Together with some volutids (i.e., *Adelomelon*, *Zidona*) and nassariids (i.e., *Buccinanops*), *Olivancillaria* species are among of the most common endemic taxa living in sandy bottoms of the Argentine malacological province.

In contrast to many other neogastropod families, the ultrastructure of the sperm of the Volutidae and Olividae in the southwestern Atlantic Ocean has not been intensively examined, except for Giménez et al., 2008; Zabala et al., 2009. Other studies have relied on traditional anatomical morphology, with most literature on these families still focused on their systematics (Marcus and Marcus, 1959; Klappenbach, 1965, 1966; Weaver and du Pont, 1970; Novelli and Novelli, 1982; Darragh, 1988; Poppe and Goto, 1992; Bondarev, 1995; Bail and Poppe, 2001; Pastorino, 2003; Absalão and Pimenta, 2003).

The caenogastropod testis typically produces two types of spermatozoa: eusperm and parasperm. The structures of both morphological forms of sperm contribute to an understanding of the reproductive biology of these animals, and have also been useful in elucidating the taxonomic and phylogenetic relationships among them (Ponder et al., 2007).

The following account describes the ultrastructure of the euspermatozoa of two neogastropod species, the volutid *Adelomelon beckii* and the olivid *Olivancillaria deshayesiana*, and identifies several features of potential systematic importance.

MATERIALS AND METHODS

Reproductively mature males of *Adelomelon beckii* and *Olivancillaria deshayesiana* were trawled off Mar del Plata, Argentina (38°20′ S, 57°37′ W) (Figure 1) at depths of 35–40 m and 8–12 m, respectively. Small pieces of the testis were fixed in 2% glutaraldehyde in phosphate buffer [0.1 M, pH 7.0] for 4 hours at 4°C. Subsequently, the tissue pieces were placed in a 1% solution of osmium tetroxide (in 0.1M phosphate buffer) for 1.5 h and washed in buffer. Tissues were dehydrated using an ascending series of ethanol concentrations (20% to absolute ethanol), placed in a 1:1 ethanol: propylene oxide solution for 15 min and embedded in Spurr's epoxy resin. Ultrathin sections were cut using either a Reichert or an LKB IV ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds, 1963). All sections were examined and photographed using Zeiss (Oberkochen, Germany) EM 109T, Hitachi 300 and Jeol 1010 transmission electron microscopes operated at 75–80 kV.

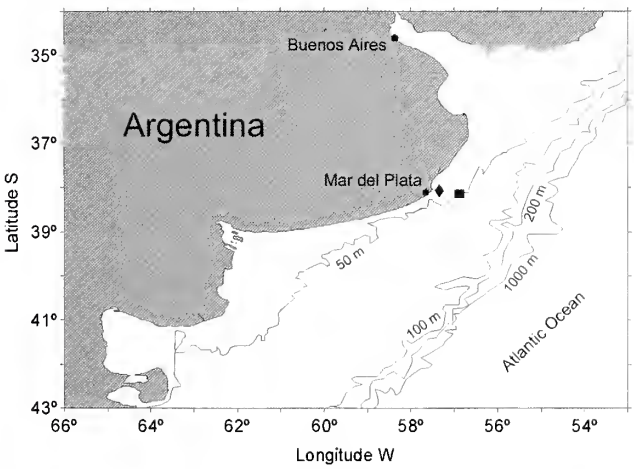


Figure 1. Map showing presence of the studied species in sampled sites ♦ = *Olivancillaria deasayesiana* location and ■ = *Adelomelon beckii* location.

RESULTS

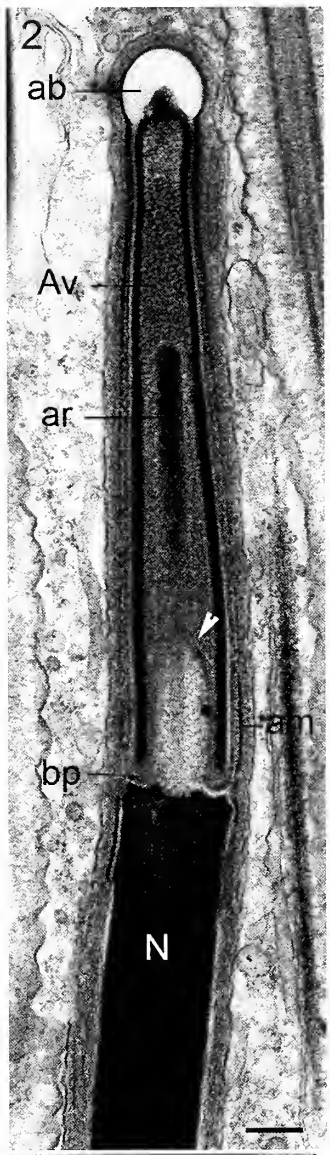
The euspermatozoa of *Adelomelon beckii* and *Olivancillaria deshayesiana* share the same general morphology, being composed of an acrosomal complex, nucleus, midpiece, glycogen piece, and end piece.

Acrosomal Complex: The acrosomal complex consists of a tall-conical, membrane-bound acrosomal vesicle, an axial rod and a basal plate (Figures 2, 14). The acrosomal vesicle is approximately 7.39 ± 0.95 µm long in *A. beckii* and 0.47 ± 0.018 µm long in *Olivancillaria deshayesiana*. Apically, the vesicle membrane is separated from the vesicle contents by the apical bleb. The acrosomal vesicle bears a very deep invagination that contains the axial rod (subacrosomal material). In *Adelomelon beckii* and *O. deshayesiana*, longitudinal sections show a constriction of this invagination. These constrictions measure 1.26 ± 0.26 µm in *A. beckii* and 0.20 µm in *O. deshayesiana*. An accessory membrane is closely associated with the base of the acrosomal vesicle in *A. beckii* but not in *O. deshayesiana*. The acrosomal vesicle is oval in transverse section near its base, but is laterally compressed within the apical bleb (Figures 3, 4, 5).

Nucleus: The nucleus in both species is filiform and highly electron-dense (Figures 6, 7, 15, and 16). The basal invagination contains a centriolar derivative that is continuous with the initial portion of the 9 + 2 microtubule pattern axoneme (Figures 8, 17). The length of this basal invagination is 2.46 ± 0.03 and 0.38 ± 0.05 µm in *Adelomelon beckii* and *Olivancillaria deshayesiana*, respectively.

Midpiece: Posterior to the nucleus, the axoneme is enclosed in a mitochondrial sheath to form the midpiece region. Oblique longitudinal sections show that the mitochondrial elements are disposed helically (Figures 7, 19). In *Adelomelon beckii*, an electron-dense, U-shaped outer layer is observed in the mitochondrial elements (Figures 9, 10) that is not evident in *Olivancillaria deshayesiana* (Figure 18).

Annular Complex and Glycogen Piece: An annular complex is located at the immediate junction of the midpiece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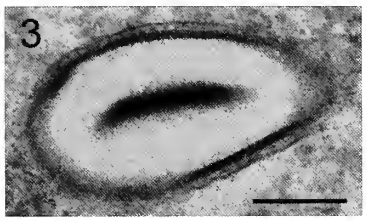
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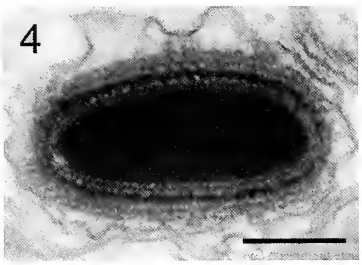
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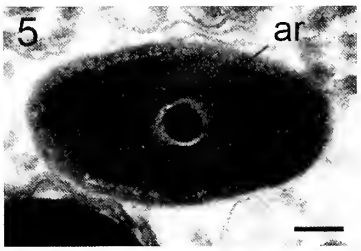
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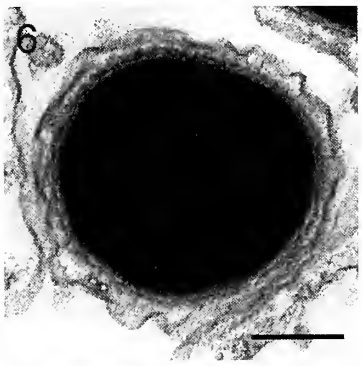
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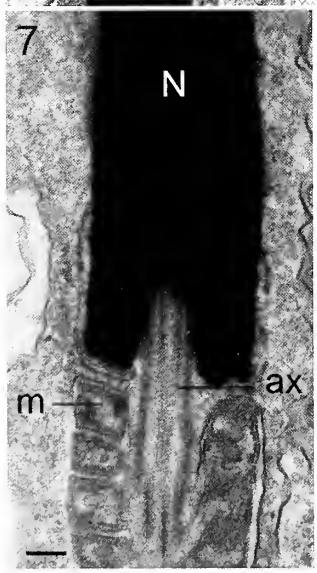
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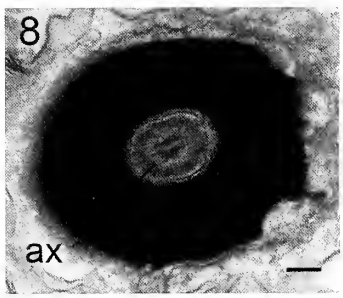
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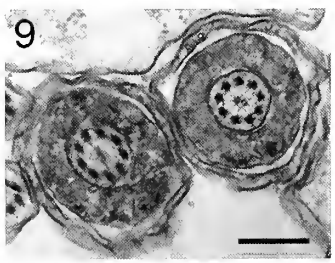
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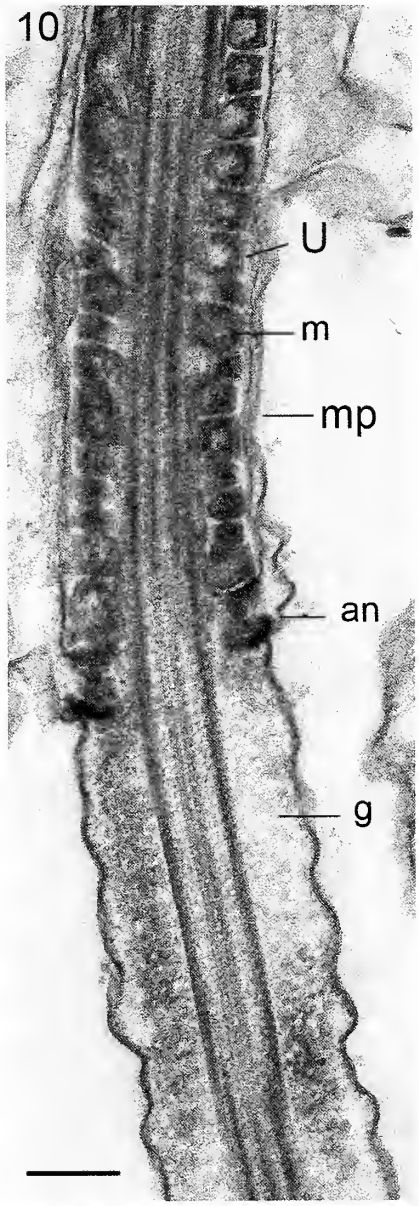
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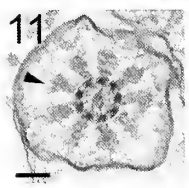
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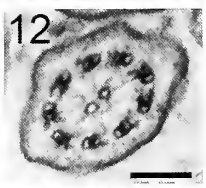
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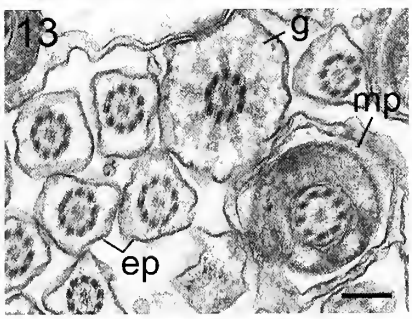
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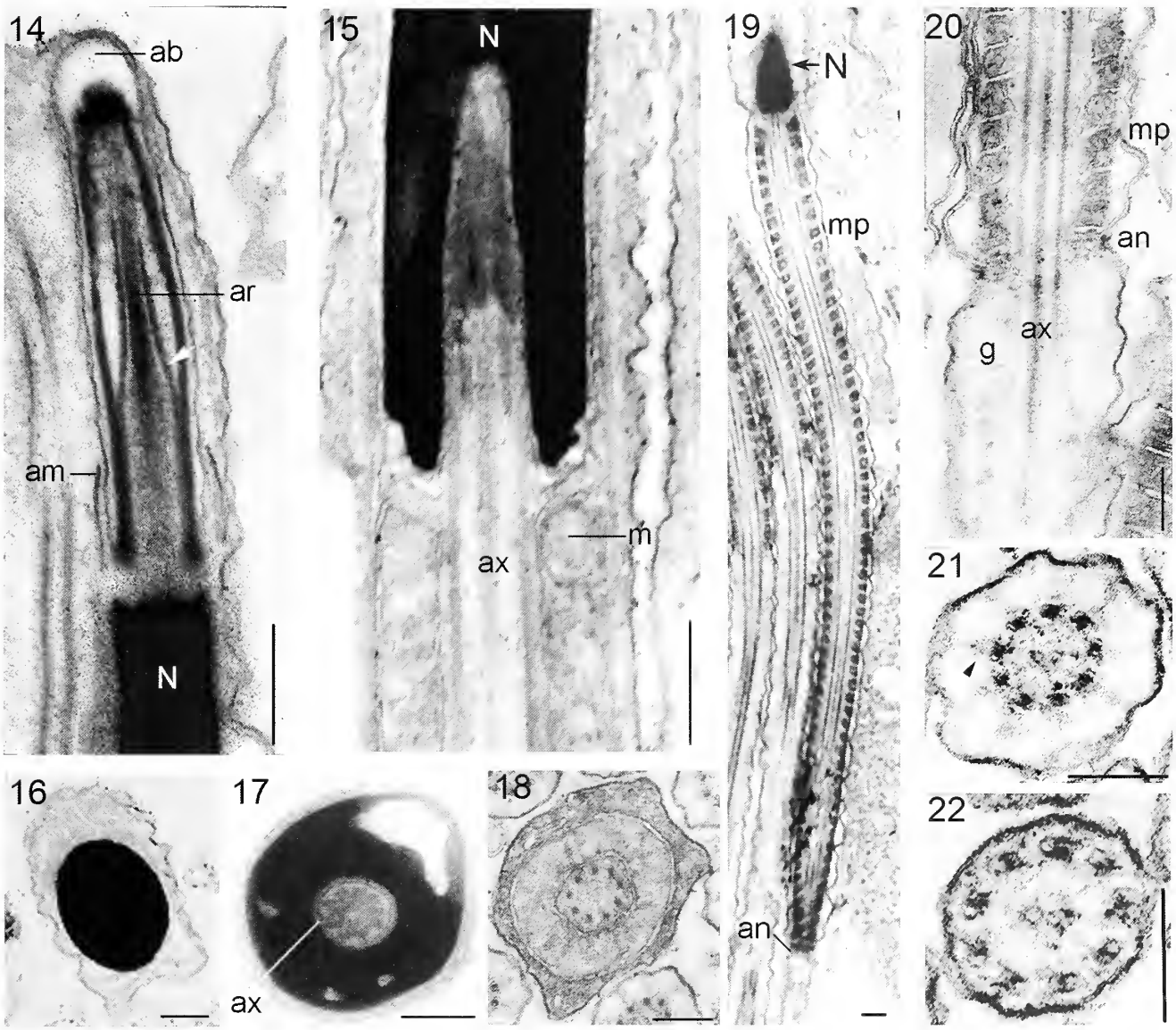

Figures 14–22. Euspermatozoa of *Olivancillaria deshayesiana*. **14.** Longitudinal section (LS) through the acrosomal complex showed the apical bleb (**ab**), the axial rod (**ar**), the accessory membrane (**am**) and the anterior portion of nucleus (**N**). **15.** LS through the nucleus (**N**) and anterior portion of midpiece with mitochondria (**m**) spiraling around the axoneme (**ax**). **16.** TS through the nucleus. **17.** TS through the nucleus with axoneme (**ax**). **18.** TS through the midpiece. **19.** LS through the midpiece (**mp**) showing the nucleus (**N**) and the annular complex (**an**). **20.** LS through the junction of the midpiece (**mp**) and glycogen piece (**g**). Note the annular complex (**an**) and the axoneme (**ax**). **21.** TS through the glycogen piece showing radiating and longitudinal rows (**arrowhead**). **22.** TS through the endpiece. Scale bars = 0.10 μm.

Figures 2–13. Euspermatozoa of *Adelomelon beckii*. **2.** Longitudinal section (LS) through the apical bleb (**ab**), the constriction (arrowhead) of the acrosomal vesicle (**Av**) and the axial rod (**ar**) in the acrosomal complex. Note the presence of the accessory membrane (**am**), the basal plate (**bp**) and the nucleus (**N**). Scale bar = 0.58 μm. **3–5.** Series of transverse sections (TS) at different levels of the acrosomal complex: **3.** The apical bleb; **4.** The middle of the acrosomal vesicle; and **5.** The axial rod (**ar**) in the region of the invagination of the acrosomal vesicle. Scale bar = 0.10 μm. **6.** TS section through the nucleus. Scale bar = 1.0 μm. **7.** LS through the junction of nucleus (**N**) and anterior portion of midpiece, showing mitochondria (**m**) spiraling around the axoneme (**ax**). Scale bar = 0.35 μm. **8.** TS of nucleus with axoneme (**ax**). Scale bar = 0.23 μm. **9.** TS of midpiece. Scale bar = 0.58 μm. **10.** LS at the junction between the midpiece (**mp**) and glycogen piece (**g**). Note the presence of the annular complex (**an**). The helical mitochondria (**m**) elements are defined by dense U-shaped profiles (**U**). Scale bar = 0.58 μm. **11.** TS through the glycogen piece showing radiating rows of putative glycogen granules (arrowhead). Scale bar = 0.58 μm. **12.** TS through the end piece. Scale bar = 0.10 μm. **13.** TS showing midpiece (**mp**), glycogen piece (**g**) and end pieces (**ep**). Scale bar = 0.58 μm.

and glycogen piece in both species studied (Figures 10, 20). Beyond the midpiece, the axoneme is associated with nine longitudinal, radiating tracts of dense granules within the glycogen piece (Figures 11, 21).

End Piece: This region of the eusperm is situated posterior to the glycogen piece, and consists of the axoneme, with a 9+2 pattern of microtubules surrounded by a plasma membrane. The diameter of the end piece is 0.70 ± 0.06 µm in *Adelomelon beckii*, and 0.16 ± 0.02 µm in *Olivancillaria deshayesiana* (Figures 12, 13, 22).

DISCUSSION

In this paper we report new and preliminary information about the ultrastructure of the sperm of one species of the family Volutidae and one species of Olividae. Our study indicates that the euspermatozoa of *Adelomelon beckii* and *Olivancillaria deshayesiana* are similar to the euspermatozoa type 2 described by Healy (1996). This eusperm presents an acrosomal vesicle with an apical bleb and accessory membrane, a solid, electron-dense nucleus a midpiece with mitochondrial elements helically coiled around the axoneme, a glycogen piece with nine tracts of granules, and a dense ring structure at the midpiece-glycogen piece junction. In *A. beckii*, the outer layer of each mitochondrial element is considerably more electron-dense than in *O. deshayesiana*. The outer layer, with its bilaminar appearance, possibly represents a partial "crystallization" of the mitochondrial elements, analogous to that occurring in certain rissoidean caenogastropods (Healy, 1983). This particular U-shaped profile of each mitochondrion is very distinctive and has not been observed in any study of caenogastropod euspermatozoa, except in three members of the family Volutidae: *Zidona dufresnei* (Donovan, 1823), *Provocator mirabilis* (Finlay, 1926) (both Giménez et al., 2008), and *Adelomelon ancilla* (Lightfoot, 1786) (Zabala et al., 2009). The glycogen pieces and end pieces of these three species and *A. beckii* and *O. deshayesiana* are the same as those observed in other caenogastropods, and show the characteristic axoneme of the group (Giménez et al., 2008; Zabala et al., 2009).

The size structure pattern of the acrosomal complex in the mature eusperm from *Olivancillaria deshayesiana* is very small when compared to the acrosomal complex of *Adelomelon beckii*. We suggest the existence of a correlation between the size of individual animals and the length of the acrosomal complex, but additional studies are needed to confirm this observation.

In *Adelomelon beckii*, the constriction in the acrosomal vesicle invagination is situated at 0.2 of the acrosomal length, measured from the posterior margin of the acrosomal complex, while in *Olivancillaria deshayesiana* the constriction is at 0.4 of the acrosomal length. We postulate that the relative position of the constriction of the acrosomal vesicle relative to the length of the acrosomal complex may be of systematic significance. Additional sampling is required to determine if these values are diagnostic of the families Volutidae and Olividae, and if this character has broader utility in clarifying phylogenetic relationships within the Neogastropoda.

ACKNOWLEDGMENTS

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Spawn characteristics in *Adelomelon ferussacii* (Donovan, 1824) (Gastropoda: Volutidae) from southern Patagonia, Argentina

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ABSTRACT

South Ameriean volutids are very homogeneous with regard to their reproductive mode. These gastropods generally spawn egg capsules containing few eggs; the embryos feed on substances contained in the intracapsular fluid and hatch as erawling juveniles. *Adelomelon ferussacii* lives on subtidal mud or sandy bottoms, yet the egg capsules collected in San Julián, Santa Cruz, Argentina, were found on flat smooth subtidal rocks. The egg capsule is globose and hemispherical, flexible, opaque-white, and the attachment base is wide, measuring between 15–30 mm in diameter. One to six eggs were recorded inside each egg eapsule. The embryonie development oceurred in the interior of the eapsule and eight stages are described. Crawling juveniles, with shells measuring between 11.25–14.8 mm, were observed at the last stage before hatching. Also a gregarious spawning event is recorded for the first time in the South American volutes.

Additional keywords: Neogastropoda, egg eapsules, development, hatching size, gregarious spawning

INTRODUCTION

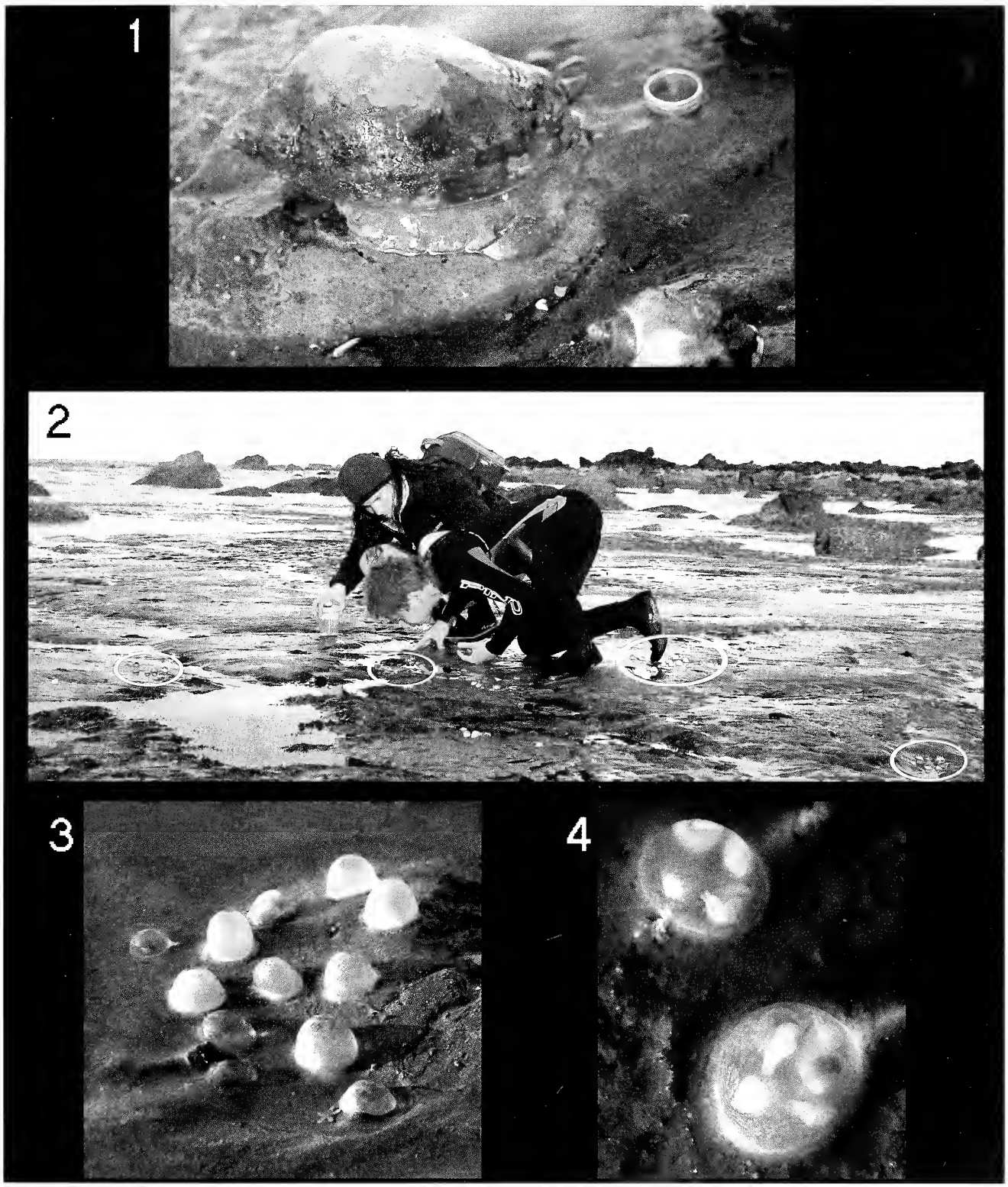
South American volutids are relatively homogenous with regard to their reproductive biology. Female volutes produce single, large egg capsules with relatively few eggs that are attached to hard substrates. Embryos develop until metamorphosis and hatch as crawling juveniles. Juveniles usually exceed 10 mm in total shell length, originating from eggs smaller than 300 µm that are suspended with extra-vitelline substances such as albumen in the intraeapsular liquid (Penchaszadeh and De Mahieu, 1976). Yet, there are exceptions, sueh as *Volnta virescens* (Lightfoot, 1786), which is reported to spawn egg capsules containing about 200 eggs. Of these, only one or two develop further, ingesting the others as nurse eggs (Bandel, 1976).

The spawnings of three species of the genus *Adelomelon* in the southwestern Atlantic have been described to date. *Adelomelon brasiliana* (Lamarck, 1811) has the largest known unattached caenogastropod egg capsules, with diameters between 40–80 mm and internal volumes of up to 140 ml (Penehaszadeh and De Mahieu, 1976). *Adelomelon ancilla* (Lightfoot, 1786) have oval and flat egg capsules, which are attached to hard substrates. The minor and major axes of their bases measure between 25–44 mm and 27–46 mm, respectively, and their internal volumes may reaeh four milliliters (Penchaszadeh et al., 1999). *Adelomelon beekii* (Broderip, 1836) have globose hemispherieal egg eapsules that are also attached to hard substrates, usually the external surfaees of empty seallop shells. Egg eapsules measure approximately 50 mm in basal diameter and have internal volumes between 30–35 ml (Penehaszadeh et al., 1999).

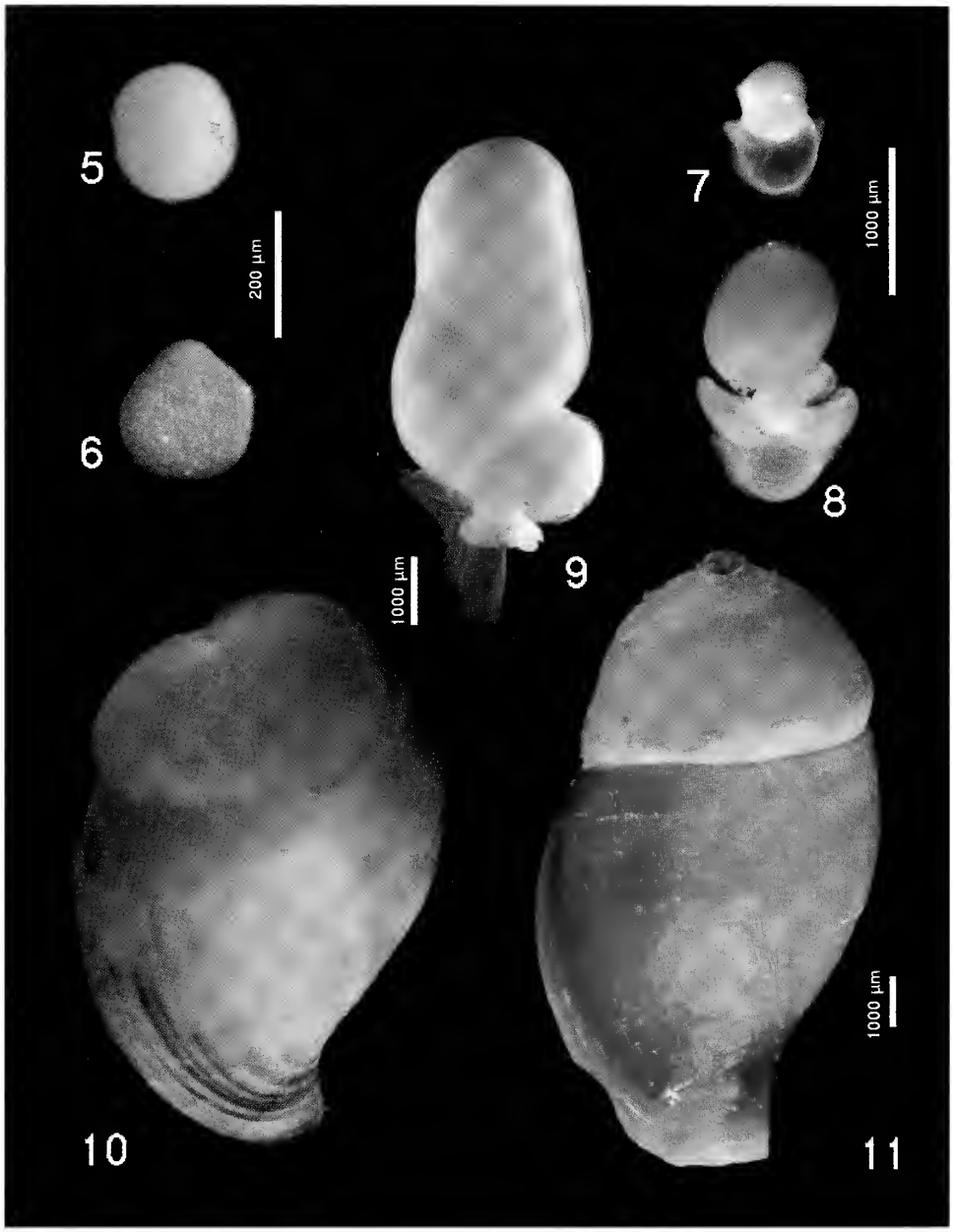
Adelomelon fernssaeii (Donovan, 1824) are distributed from 42° S (Gulf San Matias) to 52° S (Straits of Magellan) (Careelles and Williamson, 1951), corresponding to the Magellanie biogeographical province. Little is known about this speeies, whieh lives below the low water line on mud or sandy bottoms. This study deseribes the egg eapsules and the first stages of development of representatives of *A. ferussacii*.

MATERIALS AND METHODS

A total of 95 egg capsules of *Adelomelon ferussaeii* were colleeted during the austral summer by free-diving during low tides (2–3 m depth) at La Cascada in January 2005, and manually from areas exposed during an extraordinary low tide event in February 2006 at La Mina Beach, both located in San Julián, Santa Cruz Province (respeetively 67°43′ W, 49°19′ S and 67°40′ W, 49°15′ S). The water temperature at the time of eollection was 15°C. Egg eapsules were collected from the roeky bottom by hand with the help of a spatula, preserved in individual jars in 70 % ethanol, and examined under dissecting and transmission optieal microscopes, as needed. The diameter and height of each egg capsule was measured using a Vernier ealiper, and each internal volume was measured by carefully extracting the intracapsular liquid with a Pasteur pipette. Egg capsules were opened by eutting along their base line using a small surgical scissors. The number and stage of eaeh



Figures 1–4. Habitat and egg capsules of *Adelomelon ferussacii*. **1.** Adult at low tide. **2.** Panoramic view of "Playa La Mina": egg capsules of *A. ferussacii* are exposed during low tide. Circles indicate clusters of egg capsules. **3.** Detail of an egg capsule cluster. **4.** Egg capsules with juveniles close to hatching. Photographed by Natalie Collm.



Figures 5–11. Development of *Adelomelon ferussacii*. **5.** Uncleaved egg. **6.** Morula stage. **7.** First "veliger" stage. **8.** Second "veliger" stage. **9.** Late embryo without shell. **10.** Late embryo with calcified shell. **11.** Pre-juvenile close to hatching.

embryo was recorded and photographs of each stage were obtained through the microscope.

RESULTS

Spawning Sites: *Adelomelon ferussacii* (Figure 1) lays egg capsules on rocky bottoms. At La Cascada, where there is sandy-mud bottom, they were attached to flagstone slabs. At La Mina Beach, egg capsules were attached to the flat rocky bottom. Communal spawning was observed at both sites. The aggregation of spawning females results in a spawn cluster of more than 20 egg capsules (Figure 2), which indicates a gregarious behavior for spawning. Within the spawn cluster, individual egg capsules showed different developmental stages.

Characteristics of the Egg Capsule: The spawn consists of a single egg capsule attached to hard substrate, either a flagstone slab or another type of flat, rocky substrate. The egg capsule is globose, hemispherical and flexible, with a white opaque color (Figures 3, 4). It had a basal minor axis measuring 15–18 mm (N = 95), basal major axis measuring 29–31 mm (N = 95), and height of 11–21 mm (N = 95). The internal volume of egg capsules was 1.2–6.0 ml (N = 95) (Table 1). No exit plug or escape aperture was observed in

Table 1. Dimensions of the egg capsule of *Adelomelon ferussacii*.

	N	Mean	Max.	Min.	SD
Diameter (mm)	95	21 × 23	29 × 31	15 × 18	2.30 × 2.52
Height (mm)	95	15.52	21	11	2.25
Int. volume (ml)	95	2.74	6	1.2	0.89

any capsule, only a suture line on one side of the capsules. The base is round with a narrow margin (~3 mm). No external calcareous layer was present.

Characteristics of the Early Developmental Stages: Out of the 95 egg capsules collected, only 61 contained embryos. The majority of the embryos were found in late developmental stages. Between one and six embryos per egg capsule were found, with a mode of three (mean = 2.8; SD = 1.1; N = 61) (Table 2). The following stages of development were identified: uncleaved egg; eight-cells; morula; "veliger I"; "veliger II"; late embryo without shell; late embryo with shell; and pre-juveniles close to hatching (Figures 5–11). The uncleaved egg diameter was 220 µm (N = 1), the eight-cell diameter was 220 µm (N = 1), the embryos in the morula stage measured 210–240 µm diameter (mean = 224 µm; N = 5). Those embryos in "veliger I" measured 750–950 µm in length (mean = 810 µm; N = 4); "veliger II" 1250–3500 µm in length (mean = 1860 µm; N = 21); embryos without shell 5–15 mm in length (mean = 8.3 mm; N = 98). The embryos presenting calcified shells ranged between 7.5–12.5 mm total shell length (mean = 9.7 mm; N = 34) and embryos close to hatching between 11.2–14.8 mm in total length (mean = 13.1 mm; N = 9) (Table 3).

DISCUSSION

Information on the spawning of volutids is scarce not only for South American species, but also for those from other regions of the world. As a general rule, South American volutids show little variation with regard to their reproductive patterns. Commonly, the egg capsules are attached to hard substrates; the fact that *Adelomelon brasiliana* spawns free eggs capsules is a remarkable adaptation to shallow sandy bottoms, given that they may

Table 2. Frequency of number of embryos per egg capsule in *Adelomelon ferussacii* collected in January 2005 and February 2006 in "La Cascada" and "La Mina" beach, San Julian, Argentina (N = 95).

N° embryos	Frequency
0	34
1	7
2	15
3	24
4	12
5	2
6	1
more	0

Table 3. Size at different stages of development identified for *Adelomelon ferussacii*.

Stages	n	Mean
Uncleaved egg	1	220 µm
8 cells	1	220 µm
Morule	5	224 µm
Veliger I	4	0.8 mm
Veliger II	21	1.8 mm
Embryo without shell	98	8.3 mm
Embryo with shell	34	9.7 mm
Close to hatching	9	13.0 mm

be carried away by the currents but are never buried in the sand (Penchaszadeh and De Mahieu, 1976).

Adelomelon ferussacii lives in shallow water, on mixed or soft bottoms along the Magellanic biogeographical province. The only available information on this species is based on very few specimens and mainly on shell features (e.g., Clench and Turner, 1964; Weaver and du Pont, 1970). As with all the other studied South American volutids, except for a single report on *Voluta virescens* (Bandel, 1976), *Adelomelon ferussacii* spawns egg capsules containing few eggs. The embryos feed on substances contained in the intracapsular fluid. Development is direct (intracapsular metamorphosis) and crawling hatchlings may have a shell length of more than 10 mm (Carcelles, 1944; De Mahieu et al., 1974; Penchaszadeh and De Mahieu, 1976; Penchaszadeh, 1988; Hain, 1992; Penchaszadeh et al., 1999).

The diameter of the eggs of *Adelomelon ferussacii*, including the uncleaved egg, eight-cells and morula stages, is about 220 µm. This size is smaller than the egg sizes reported for *Voluta musica* Linnaeus, 1758 (330 µm) (Penchaszadeh and Miloslavich, 2001). However, the egg size we measured is similar to those sizes reported for *Adelomelon brasiliana* (Lamarck, 1811) (240 µm), *A. ancilla* (Lightfoot, 1786) (200–220 µm) (Penchaszadeh and De Mahieu, 1976), and *Odontocymbiola magellanica* (Gmelin, 1791) (210 µm) (Bigatti, 2005), but larger than those of *Zidona dufresnei* (Donovan, 1823) (90 µm) (Penchaszadeh and de Mahieu, 1976).

The embryological development is similar to those described for *A. brasiliana* and *A. ancilla* by Penchaszadeh and de Mahieu (1976), with presence of a poorly developed velum. This contrasts with *Voluta musica* Linnaeus, 1758, which has a well-developed and wide intracapsular velum, the largest of the studied volutids (Penchaszadeh and Miloslavich, 2001).

Gastropod egg capsules are morphologically and chemically complex; they provide mostly protection against bacterial attacks, environmental stress, and predation (Pechenik 1979, 1986; Miloslavich 1996). Despite this, studies show that gastropod egg capsules are targets for predation by fish, crustaceans, polychaetes, and even other gastropods (D'Asaro, 1970). In this study, preyed-upon *Adelomelon ferussacii* egg capsules were observed. These were found lacerated mainly on their upper portions, probably by sea birds such as *Larus dominicanus* (Lich-

tenstein, 1823) and *Haematopus ater* Vieillot and Oudart, 1825, which were observed pecking on the egg capsules when these were exposed at low tides. Bird predation on volute stranded free egg capsules (*Adelomelon brasiliana*) was studied by Penchaszadeh et al. (2000).

Adelomelon ferussacii egg capsules lack an external calcium carbonate cover such as found in the common Patagonian *Odontocymbiola magellanica* (see Bigatti, 2005); this would increase their susceptibility to predation.

Gregarious behavior was observed for the spawning of *A. ferussacii*, as has been reported for several caenogastropod species such as *Engoniophos unicinctus* Say, 1825 (Miloslavich and Penchaszadeh, 1994), *Fusinus closter* Philippi, 1850 (Miloslavich and Penchaszadeh, 1997), and *Chicoreus margaritensis* (Abbott, 1958) (Cipriani, 1990). This conclusion is based on observations of presence of patches of egg capsules in different developmental stages along the shore. This is, to our knowledge, the first report of this behavior in the family Volutidae.

ACKNOWLEDGMENTS

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The relationships of the enigmatic gastropod *Tritonoharpa* (Neogastropoda): New data on early neogastropod evolution?

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ABSTRACT

In this paper, the relationships of *Tritonoharpa* Dall, 1908, within Neogastropoda are discussed. *Tritonoharpa* is indeed similar to *Colubraria* in the morphology of its head-foot, pallial complex, reproductive and excretory systems, in the presence of an extremely long and coiled proboscis, and a very large stomach. However, it differs from *Colubraria* in the rest of its foregut anatomy, revealing a cancellariid affinity, and a typical nematoglossan radula. The molecular data confirms Beu and Maxwell's placement of *Tritonoharpa* in the Cancellariidae, close to *Plesiotriton*. It is also suggested that cancellariids may be the sister-group to the rest of neogastropods. *Tritonoharpa* has a rather large and well developed midgut gland, resembling the gland of Leiblein. As previously studied cancellarioideans have been shown to lack a well differentiated gland of Leiblein, the present study raises some interesting questions about the evolution of the foregut in Neogastropoda. In fact, if this glandular structure were confirmed as a true homologue of the gland of Leiblein, and the cancellarioideans proved to be the sister group to the remaining neogastropods, the possession of the gland should be considered a synapomorphy of the Neogastropoda.

Additional keywords: Anatomy, phylogeny, molecular systematics, Neogastropoda, Cancellariidae

INTRODUCTION

Tritonoharpa antiquata (Hinds in Reeve, 1844) belongs to a small group of 19 Recent species, most occurring in the tropical Indo-West Pacific (Beu and Maxwell, 1987). These species had previously been referred to a *Colubraria*-like group, together with members of at least four families (Beu and Maxwell, 1987). Elongate and varicate shells, typical of *Colubraria*, have evolved through convergence several times in the families Ranellidae, Muricidae, Buccinidae, and Cancellariidae. A number of genera with columellar plaits and a nematoglossan radula, morphologically similar to *Plesiotriton*, Fisher, 1884, were placed in the Cancellarioidea. Among those, the genus *Tritonoharpa* Dall, 1908 (type species by original designation, *Tritonoharpa vexillata* Dall, 1908, Recent, from western America and the Galapagos Islands) was distinguished from *Plesiotriton* only by the absence of columellar plaits and the absence of radula (Beu and Maxwell, 1987).

Information on the anatomy of Cancellariidae is available (Harasewych and Petit, 1982; 1984; 1986), based on representatives of the subfamilies Cancellariinae and Admetinae. The anatomy and phylogenetic relationships of the Plesiotritoninae to the other cancellariids are still unknown.

Herein we describe the foregut anatomy of *Tritonoharpa antiquata* (Figure 18) and compare it with anatomical data already available for other cancellariids. A molecular dataset, based on two mitochondrial markers (12S and 16S rDNA) was used to construct a molecular phylogenetic framework for the systematics of the Plesiotritoninae.

MATERIALS AND METHODS

Taxon Sampling and Specimen Collection: The material for the present study was collected during field work and expeditions to the West Pacific (Panglao 2004, Philippines, and Santo 2006, Vanuatu, organized by the Muséum national d'Histoire naturelle, Paris), Panama (Neogastropod Workshop 2006 at the Smithsonian Tropical Research Institution, Panama), the Mediterranean Sea, and other localities, and supplemented by specimens provided by Museums and colleagues (see Table 1 for details). Vouchers are stored at BAU (Department of Animal and Human Biology, Rome), MNHN (Muséum national d'Histoire naturelle, Paris), NMSA (Natal Museum, Pietermaritzburg).

Representatives of 21 additional neogastropods, including representatives of 13 families were sequenced to provide a phylogenetic framework for the relationships of *Tritonoharpa* to other cancellariids and within the Neogastropoda. The cypraeid *Cypraca cervinetta* Kiener, 1843 has been chosen as an outgroup (see Table 2 for details).

Table 1. Species included in the molecular analysis, with collecting data, voucher numbers, length of the 12S and 16S sequences, and EMBL accession numbers. BAU, Department of Animal and Human Biology, Rome; MNHN, Muséum National d'Histoire Naturelle, Paris; NMSA, Natal Museum, Pietermaritzburg; and EMBL, The European Molecular Biology Laboratory, Heidelberg.

				12S		16S		
Family	Species	Locality	Voucher Number	EMBL	bp	EMBL	bp	References
Cypraeidae	*Cypraea cervinetta* Kiener, 1843	Venado (Panama), 8.89° N, 79.59° W, intertidal	BAU00799	FM999072	521	FM999103	492	Oliverio and Modica, in press
Cancellariidae	*Cancellaria cancellata* Linné, 1767	Off Malaga (Spain), 40–50 m	BAU00224	FM999074	541	FM999105	652	Oliverio and Modica, in press
Cancellariidae	*Cancellaria cooperi* Gabb, 1865	Off La Jolla (California, USA), 40 m	MNHN IM-2009-4611 BAU00797	FM999073	537	FM999104	616	Oliverio and Modica, in press
Cancellariidae	*Tritonoharpa antiquata* (Hinds in Reeve, 1844)	Mactan Is. (Philippines), 10.32° N, 124.03° E, 40–120 m, tangle nets, 15 May 2006	BAU00270	FN392228	521	FN392229	489	This work
Cancellariidae	*Plesiotriton vivus* Habe and Okutani, 1981	Bohol/Sulu sea sill (Philippines), PANGLAO 2005, st CP2359, 8.83° N 123.58° E, 437–476 m	MNHN32123	FM999075	523	FM999106	656	Oliverio and Modica, in press
Conidae	*Conus textile* Linnaeus, 1758	Philippines	–	DQ862058	535	DQ862058	609	Bandyopadhyay et al., 2007
Turridae	*Lophiotoma cerithiformis* Powell, 1964	Philippines	–	DQ284754	532	DQ284754	625	Bandyopadhyay et al., 2006
Muricidae	*Nucella lapillus* Linnaeus, 1758	Portobello (UK), 55.95° N, 3.10° W, intertidal	MNHN IM-2009-4617 BAU00187	FM999088	527	FM999119	679	Oliverio and Modica, in press
Muricidae	*Cronia* sp. 1	Tolo Channel, Hong Kong, 22.45° N, 114.26° E, 1 m depth	MNHN IM-2009-5118 BAU00619	FN391982	521	FM999120	669	Oliverio and Modica, in press
Muricidae	*Stramonita haemastoma* (Linné, 1767)	S. Marinella (Italy), 42.0° 3 N, 11.90° E, intertidal	BAU00696	FM999090	525	FM999121	661	Oliverio and Modica, in press
Muricidae	*Drupella cornus* Röding, 1798	Panglao Is., Catarman (Philippines), PANGLAO 2004, st. R18, 9.60° N, 123.86° E, 2–46 m	MNHN IM-2009-4601 BAU00192	FM999091	521	FM999122	657	Oliverio and Modica, in press
Buccinulidae	*Paraeuthria plumbea* (Philippi, 1841)	Ushuaia (Argentina), 54.78° S, 68.23° W, intertidal	MNHN IM-2009-4613 BAU00697	FM999095	530	FM999126	637	Oliverio and Modica, in press
Buccinidae	*Neobuccinum eatoni* (Smith, 1875)	Terra Nova Bay (Antarctic), 74.69° S, 164.1° 2 E	MNHN IM-2009-4614 BAU00785	FM999096	535	FM999127	657	Oliverio and Modica, in press
Nassariidae	*Ilyanassa obsoleta* (Say, 1822)	Not available		DQ238598	535	DQ238598	563	Simison et al., 2006
Nassariidae	*Nassarius pagodus* (Reeve, 1844)	Las Perlas Is. (Panama), 8.74° N, 79.20° W, 50 m	MNHN IM-2009-4620 BAU00237	FM999094	528	FM999125	659	Oliverio and Modica, in press
Melongenidae	*Melongena patula* (Broderip and Sowerby, 1829)	Venado (Panama), 8.89° N, 79.59° W, intertidal	MNHN IM-2009-4621 BAU00794	FM999093	533	FM999124	671	Oliverio and Modica, in press
Melongenidae	*Volema myristica* (Röding, 1798)	Panglao Is., Sungcolan (Philippines) PANGLAO 2004, st, M11, 9.64° N, 123.83° E, 0–3 m	MNHN IM-2009-4602 BAU00225	FM999091	534	FM999123	662	Oliverio and Modica, in press

Olividae	*Oliva spicata* (Röding, 1798)	Las Perlas (Panama), 8.53° N, 79.09° W, 20–22 m	MNHN IM-2009-4616 BAU00278	FM999083	524	FM999114	672	Oliverio and Modica, in press
	Olivella volutella (Lamarck, 1811)	Venado (Panama), 8.89° N, 79.59° W, intertidal	MNHN IM-2009-4615 BAU00241	FM999082	534	FM999113	665	Oliverio and Modica, in press
Pseudolividae	*Sylvanocochlis ancilla* (Hanley, 1859)	SW of Mossel Bay, Agulhas Bank, Western Cape (South Africa), 81 m	NMSA-E5279	FM999084	532	FM999115	489	Oliverio and Modica, in press
Costellariidae	*Vexillum plicarium* (Linnaeus, 1758)	Panglao Is.,Tangibilaran-Panglao Channel (Philippines), Panglao-2004, st. R67, 9.64° N , 123.86° E, 3.0–3.5 m	MNHN IM-2009-4603 BAU00207	FM999081	535	FM999112	489	Oliverio and Modica, in press
Volutomitridae	*Microvoluta* sp.	Bohol/Sulu Seas sill (Philippines), Panglao 2005 St. CP2358, 8.87° N, 123.62° E, 569–583 m	MNHN IM-2009-4609 BAU00699	FM999080	525	FM999111	651	Oliverio and Modica, in press
Ptychatractidae	*Latiromitra* sp.	Bellona West (New Caledonia), Coral Sea, Ebisco, st. CP2556, 21.1° S, 158.53° E, 741–791 m	MNHN IM-2009-4610 BAU00612	FM999085	525	FM999116	653	Oliverio and Modica, in press

Table 2. The specimens of *Tritonoharpa antiquata* with their shell measurements (in mm) and their use in this study. Abbreviations: **H,** shell length; **h,** length of the last whorl: **al,** aperture length.

Specimen/Voucher ID	locality	H	w	h	al	Sex	
BAU00268	Aliguay Is. (Philippines), 8.75° N, 123.23° E, 30–150 m, tangle nets, May 2006	15.7	5.4	9.4	6.8	male	dissected
BAU00269	Aliguay Is. (Philippines), 8.75° N, 123.23° E, 30–150 m, tangle nets, May 2006	18.5	6.1	9.9	7.2	female	dissected
BAU00270	Mactan Is. (Philippines), 10.32° N, 124.03° E, 40–120 m, tangle nets, 15 May 2006	14.1	4.6	8.4	6	female	DNA
BAU00301	Santo Is. (Vanuatu), Santo 2006, sta. DR74, SE Matewulu, 15.38° S, 167.19° E, 6 m (J. Pelorce leg.)	15.3	5.1	9.1	6.6	female	dissected
BAU00302	Santo Is. (Vanuatu), Santo 2006 sta. DR74, SE Matewulu, 15.38° S, 167.19° E, 6 m (M. Oliverio leg.)	20	6.5	10.3	8.1	female	sectioned
BAU00303	Santo, Vanuatu, Santo 2006 sta. DR55, Palikulo Bay, 15.48° S, 167.25° E, 3–7 m (J. Pelorce leg)	19.1	6.3	10.1	7.3	female	dissected

Table 1. Species included in the molecular analysis with collecting data, voucher numbers, length of the 12S and 16S sequences, and EMBL accession numbers. BAU: Department of Animal and Human Biology, Rome; MNHN: Museum National d'Histoire Naturelle, Paris; NMSA: Natal Museum, Pietermaritzburg; and EMBL: The European Molecular Biology Laboratory, Heidelberg.

				12S		16S		
Family	Species	Locality	Voucher Number	EMBL	bp	EMBL	bp	References
Cypraeidae	*Cypraea cervinetta* Kiener, 1843	Veraguas (Panama), 8.89° N, 79.59° W, intertidal	BAU00799	FM999072	521	FM999103	492	Oliverio and Modica, in press
Cancellariidae	*Cancellaria cancellata* Linné, 1767	Off Malaga (Spain), 10–50 m	BAU00224	FM999071	541	FM999105	652	Oliverio and Modica, in press
Cancellariidae	*Cancellaria reticulata* Gabb, 1865	Off La Jolla (California, USA), 10 m	MNHN IM-2009-4611 BAU00797	FM999073	537	FM999104	646	Oliverio and Modica, in press
Cancellariidae	*Tritonoharpa antiquata* (Hinds in Reeve, 1844)	Mactan Is. (Philippines), 10.32° N, 124.03° E, 40–120 m, tangle nets, 15 May 2006	BAU00270	FN392228	521	FN392229	489	This work
Cancellariidae	*Plesiotriton crenulatus* Habe and Okutani, 1981	Bohol/Sulu Sea, off Cebu (Philippines), EXPEDITO 2005, st. CP2359, 9.83° N 123.58° E, 437–476 m	MNHN32123	FM999075	523	FM999106	656	Oliverio and Modica, in press
Conidae	*Conus textile* Linnaeus, 1758	Philippines	–	DQ862058	535	DQ862058	609	Bandyopadhyay et al. 2007
Turridae	*Lophiotoma cerithiformis* Powell, 1964	Philippines		DQ284774	532	DQ284774	625	Bandyopadhyay et al. 2006
Muricidae	*Nucella lapillus* Linnaeus, 1758	Portobello (UK), 55.95° N, 3.10° W, intertidal	MNHN IM-2009-1647 BAU00187	FM999088	527	FM999119	679	Oliverio and Modica, in press
Muricidae	*Cronia* sp. 1	Tolo Channel, Hong Kong, 22.45° N, 114.26° E, 1 m depth	MNHN IM-2009-5118 BAU00619	FN391982	521	FM999120	680	Oliverio and Modica, in press
Muricidae	*Stramonita haemastoma* (Linné, 1767)	S. Marinella (Italy), 42.0° N, 11.90° E, intertidal	BAU00696	FM999090	525	FM999121	661	Oliverio and Modica, in press
Muricidae	*Dicathais orbita* (Gmelin, 1791)	Panglao Is., Catarman (Philippines), EXPEDITO 2004, st. BIS, 9.60° N, 123.86° E, 2–40 m	MNHN IM-2009-4601 BAU00192	FM999091	521	FM999122	657	Oliverio and Modica, in press
Buccinulidae	*Pareuthria plumbea* (Philippi, 1844)	Ushuaia (Argentina), 54.78° S, 68.23° W, intertidal	MNHN IM-2009-4613 BAU00697	FM999095	530	FM999126	637	Oliverio and Modica, in press
Buccinulidae	*Neobuccinum eatoni* (Smith, 1875)	Terra Nova Bay (Antarctic), 74.69° S, 164.1° E.	MNHN IM-2009-4611 BAU00785	FM999096	535	FM999127	657	Oliverio and Modica, in press
Nassariidae	*Hymenosyrinx thosdeta* (Say, 1822)	Not available		DQ238598	535	DQ238598	563	Simison et al. 2006
Nassariidae	*Nassarius pigmaeus* (Reeve, 1844)	Las Perlas Is. (Panama), 8.74° N, 79.20° W, 50 m	MNHN IM-2009-4620 BAU00237	FM999094	528	FM999125	659	Oliverio and Modica, in press
Melongenidae	*Melongena patula* (Broderip and Sowerby, 1829)	Veraguas (Panama), 8.89° N, 79.59° W, intertidal	MNHN IM-2009-4621 BAU00704	FM999093	533	FM999124	671	Oliverio and Modica, in press
Melongenidae	*Volema myristica* (Röding, 1798)	Panglao Is., Simgerdan (Philippines), EXPEDITO 2004, st. M11, 9.64° N, 123.83° E, 0–3 m	MNHN IM-2009-4602 BAU00225	FM999081	534	FM999123	662	Oliverio and Modica, in press

Olividae	*Oliva sayana* (Röding, 1798)	Las Perlas (Panama), 8.53° N, 79.09° W, 20–22 m	MNHN IM-2009-4616 BAU00278	FM999083	524	FM999114	672	Oliverio and Modica, in press
	Oliva reticularis (Lamarck, 1811)	Veraguas (Panama), 8.89° N, 79.59° W, intertidal	MNHN IM-2009-4615 BAU00241	FM999082	534	FM999113	665	Oliverio and Modica, in press
Pseudolividae	*Sykracochlis mallia* (Hadley, 1859)	SW of Mossel Bay, Agulhas Bank, Western Cape (South Africa), 81 m	NMSA-E5279	FM999084	532	FM999115	489	Oliverio and Modica, in press
Costellariidae	*Vexillum plicarium* (Linnaeus, 1758)	Panglao Is., Tangibilan-Panglao Channel (Philippines), EXPEDITO 2001, st. R67, 9.64° N, 123.86° E, 3.0–3.5 m	MNHN IM-2009-4603 BAU00207	FM999085	535	FM999112	489	Oliverio and Modica, in press
Volutomitridae	*Microvoluta* sp.	Bohol/Sulu Seas, off (Philippines), EXPEDITO 2005 St. CP2358, 8.87° N, 123.62° E, 569–583 m	MNHN IM-2009-4609 BAU00699	FM999080	525	FM999111	651	Oliverio and Modica, in press
Ptychatractidae	*Latirostra* sp.	Bellona West (New Caledonia), Coral Sea, Entrecasteaux, st. CP2556, 21.1° S, 158.53° E, 741–791 m	MNHN IM-2009-4610 BAU00612	FM999085	525	FM999116	653	Oliverio and Modica, in press

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BAU00268	Alibagay Is. (Philippines), 8.75° N, 123.23° E, 30–150 m, tangle nets, May 2006	15.7	5.4	9.4	6.8	male	dissected
BAU00269	Alibagay Is. (Philippines), 8.75° N, 123.23° E, 30–150 m, tangle nets, May 2006	18.5	6.1	9.9	7.2	female	dissected
BAU00270	Mactan Is. (Philippines), 10.32° N, 124.03° E, 40–120 m, tangle nets, 15 May 2006	14.1	4.6	8.4	6	female	DNA
BAU00301	Santo Is. (Vanuatu), SANTO 2006, sta. DR74, SE Matevulu, 15.38° S, 167.19° E, 6 m (J. Pelorce leg.)	15.3	5.1	9.1	6.6	female	dissected
BAU00302	Santo Is. (Vanuatu), SANTO 2006 sta. DR74, SE Matevulu, 15.38° S, 167.19° E, 6 m (M. Oliverio leg.)	20	6.5	10.3	8.1	female	sectioned
BAU00303	Santo, Vanuatu, SANTO 2006 sta. DR55, Palikulo Bay, 15.48° S, 167.25° E, 3–7 m (J. Pelorce leg)	19.1	6.3	10.1	7.3	female	dissected

In the Results and the Discussion seetions, we have used collective taxonomie names within quotation marks (e.g.: 'volutoid', 'bueeinoid') as descriptive terms in the traditional context of the names (e.g., Ponder, 1974), but without attributing a specific taxonomic rank to them.

Anatomical Methods: Four speeimens of *Tritonoharpa antiquata* were manually dissected (two from the Philippines BAU00268-9 and two from Vanuatu BAU00301, BAU00303). One female (from Vanuatu, BAU00302) was embedded in paraffin and serially sectioned at a thickness of 7 µm. The sections were stained either with hematoxylin and aleoholic eosin, or with hematoxylin, eosin and Alcian Blue. Radulae were cleaned in liquid bleach [NaOCl], air-dried, coated with gold, and examined using a JEOL seanning electron mieroseope.

DNA Extraction, PCR, Cloning, and Sequencing: Total DNA was extracted following a standard Phenol/Chloroform/Ethanol protoeol (Hillis et al., 1990) with slight modification as previously deseribed by Oliverio and Mariottini (2001). The QIAGEN QiAmp Extraction Kit was used for extraction of DNA from diffieult samples, according to manufacturer's instructions.

Partial sequenees of two mitochondrial genes encoding ribosomal DNA were PCR amplified. A region of the gene eneoding 16S rDNA encompassing the domains IV and V (Gutell and Fox, 1988) was amplified using primers 16SA (5′-CGCCTGTTTATCAAAAACAT-3′) (Palumbi et al., 1991) and 16SH (5′-CCGGTCTGAACTCAGATCAC-3′) (Espiritu et al., 2001) or CGLeuR (5′-TATTTAGGGCT TAAACCTAATGCAC-3′) (Hayashi, 2005). A portion of the gene encoding 12S rDNA corresponding to the domains II and III was amplified with primers 12SI (5′-TG CCAGCAGCCGCGGTTA-3′) and 12SIII (5′-GAGC GACGGGCGRTTWGTAC-3′) (Oliverio and Mariottini, 2001). Amplification conditions were as follows (30–35 cycles): 94°C for 30 seconds, 45–50°C for 30 seeonds, 72°C for 60 seconds. When a single band was obtained, the PCR product was purified using the Exo-Sap enzymatic method. In eases of persistent aspeeifie amplifieation, the PCR product was ligated into the pGEM-T-Easy vector according to manufacturer's (Promega) instruetions and then used to chemieally transform *E. coli* JM109 eells. Transformed colonies were selected by blue-white selection and clones containing the correct insert size were PCR-screened. Then, they were purified using the SIGMA miniprep kit. Purified products (amplicons and clones) were then double-strand sequenced with BigDye v. 2.0 (Applied Biosystems, Foster City, CA, USA) using the PCR primers and sequences visualized on automatic sequencer. Sequencing was performed by Macrogen Inc. (Seoul, South Korea). Chromatograms were analysed using the Staden Package (Version-1.6.0, Staden et al., 1998, 2005). All sequences have been deposited at EMBL (The European Moleeular Biology Laboratory, Heidelberg; see Table 1 for accession numbers).

Sequence and Phylogenetic Analysis: Sequences were aligned using Clustal X (Thompson et al., 1994; 1997) using the default settings, then edited manually. The aligned dataset is available from the authors upon request. Analyses of nueleotide sequenees were performed using Mega3.1 (Kumar et al., 2004). The uneorrected 'p' and the ML distanees between the sequences were ealculated. To test for the presenee of mutational saturation, uncorrected 'p' pairwise distanees, transition (Ts) and transversion (Tv) were plotted against the estimated ML distance (Nichols, 2005; Philippe et al., 1994) in DAMBE (Xia and Xie, 2001; Xia, 2000). The χ^2 test implemented in PAUP* v. 4b10 (Swofford, 2002) was used to test for base composition homogeneity of the aligned sequence data. The aligned sequenees were analysed under the assumptions of Maximum Parsimony, Maximum Likelihood (ML, Felsenstein, 1981) and with a Bayesian approaeh (Rannala and Yang, 1996), using the paekages PAUP* v. 4b10 (Swofford, 2002), Modeltest v. 3.7 (Posada and Crandall, 1998), MrModeltest v. 2.2 (Nylander, 2004), MrBayes v. 3.1.2 (Ronquist and Huelsenbeek, 2003), and Treefinder, June 2007 version (Jobb et al., 2004; Jobb, 2007). Each loeus (12S and 16S) was first analysed separately. A partition homogeneity test (Miekevieh and Farris, 1981; Farris et al., 1995a, 1995b; Cunningham, 1997), implemented as ILD test in PAUP*, was performed before combining the two loei (but see Darlu and Leeointre, 2002, and Yoder et al., 2001 for criticisms on ILD's effieiency in determining data eompatibility). The combined dataset was analyzed by MP, and partitioned ML and Bayesian analyses. ML analyses were performed by Treefinder, using for eaeh partition the substitution models ehosen after evaluation by Modeltest using the Akaike information eriterion. Base frequencies, relative rates of the six substitution types and model parameters were estimated separately for eaeh partition by the software during phylogenetie reconstruction. Confidence for the nodes was estimated in Treefinder using 1000 bootstrap replicates and eompared with the LR-ELW Edge Support (Expeeted Likelihood Weights on the Loeal Rearrangements: Strimmer and Rambaut, 2002; Jobb, 2007). A Bayesian analysis (BI) was performed to obtain posterior probabilities of branches using the software MrBayes, which adopts the Markov Chain Monte Carlo method to sample from posterior densities (Larget and Simon, 1999; Yang and Rannala, 1997). The substitution model used was estimated for eaeh partition using the software MrModeltest. Base frequeneies, the relative rates of the six substitution types and model parameters were estimated during the analysis, separately for eaeh partition (using the command 'unlink' in MrBayes). A four ehain metropolis-eoupled Monte Carlo analysis was run twiee in parallel for 10^6 generations, and trees were sampled every 1.000 generations, starting after a burn-in of 250,000 gencrations. Stationarity was eonsidered to be reached when the average standard deviation of split frequencies shown in MrBayes was less than 0.01 (Ronquist and Huelsenbeck, 2003). Bayesian posterior probabilities (BPP) of a branch were estimated as the percentage of trees (after burn-in) which showed that specifie node.

RESULTS

Anatomy of *Tritonoharpa antiquata:* External Morphology: Animal uniform cream in base color, with bright orange spots most frequently situated on surface of kidney and digestive gland (Figures 1–3). Foot (Figures 1–3, **ft**) partly contracted, with a deep propodial groove separating narrow propodium. Operculum absent in all specimens. Head small (Figure 4), on well-defined neck, with short, narrow, apparently non-retractable snout (**sn**) and pair of long, thick tentacles (**t**), each with a large black eye (**e**) on outer side of a basal swelling. Penis (Figure 7, **p**) of male (spm. No. 2) rather large, flattened, slightly widening distally, with small rounded orifice (**so**) at right upper angle.

Mantle: Mantle margin smooth (Figure 8). Siphon (**s**) short, muscular. Osphradium (**os**) occupying 1/3 of mantle length, approximately 1/10 of mantle width. Osphradium with broad axis, 2 equal rows of short lamellae. Ctenidium (**ct**) long, crescent-curved, slightly wider than osphradium, occupying almost entire mantle length. Females with broad capsular gland (**cg**) covering rectum. Female genital orifice (**fo**) small, slit-like, terminal. Area between ctenidium and capsular gland occupied by numerous high folds of hypobranchial gland (**hg**).

Digestive System: Proboscis extremely long, narrow (Figure 6, **pr**), folded within body haemocoel into > 10 coils (Figure 13, **pr**). In histological sections, proboscis wall consisting of columnar epithelium with basal nuclei (Figure 12, **ep**), a layer of circular muscles (**eml**) and a thick inner layer of longitudinal fibers (**lm**). Mouth opening large, terminal (Figure 6, **m**). Oral tube short, lined with thick cuticle (Figure 16, **etc**). Buccal mass short, thick (Figure 5, **bm**), occupying ~1/10 proboscis length, consisting of buccal musculature and folded cartilages (Figures 9, 11, 15, **crt**). Buccal mass surrounded by well-developed, cuticularized, funnel-like jaw plate (Figures 9, 15, 16 **jw, etc**), tubular anteriorly, expanded posteriorly into two small wings surrounding odontophore. Radula slightly shorter than odontophore (Figure 5, **r**), nematoglossan, consisting of a thin membrane and one central longitudinal row of rachidian teeth (Figure 19). Each tooth long, narrow (length >10×width), with three short cusps on distal end. Median cusp bearing vertical row of short secondary cusps (Figures 20, 21). Teeth closely set, distance between them approximately equal to their width.

Accessory salivary glands paired, strongly-coiled, thick-walled, tubular (Figure 5, **asg**), running parallel to buccal mass, tapering toward buccal tube, opening by two ducts (**asd**) into medial region of buccal cavity. Glands consisting of very thin layer of circular fibers and layer of tall columnar glandular epithelium with basal nuclei (Figure 16, **asg**). Lumen of gland filled with mucous secretion (staining blue with Alcian: Figure 16, **asg**). Proximal ends of accessory salivary glands fused together and connected to ventral part of proboscis wall by a strip of connective tissue (Figure 5, **cnt**). Buccal mass attached to bottom of buccal tube by multiple retractor muscles. Anterior esophagus thin-walled (Figure 5, **aoe**). Proboscis cavity containing thick proboscis nerves (Figure 5, **n**) and ducts of primary salivary glands.

Single proboscis retractor muscle running from base of proboscis to floor of body haemocoel (Figure 6, **prr**). Esophagus penetrating massive nerve ring (**nr**) then continuing ventrally. Spirally coiled valve of Leiblein (**vl**) situated within proboscis. Long midgut gland posterior to nerve ring, provisionally referred to as gland of Leiblein (Figure 6, **gl**), running along posterior part of esophagus. Gland well developed, easily recognized by its dark-brown color. Tissue of gland compact in histological sections, represented by globular cells with large nuclei and multiple granules, indicating strong apocrine secretion (Figure 16, 17, **gl**). Globular cells with large nuclei situated along septa internally dividing gland into distinct lobes. Gland filled with vesicles containing multiple secretion granules. Duct of this gland not found. Anterior aorta thick, running parallel to gland of Leiblein after passing through nerve ring. Primary salivary glands paired, whitish, tightly fused (Figure 6, **sg**), situated posterior to gland of Leiblein. In histological sections (Figure 17, **sg**), primary salivary glands appear clearly tubular, consisting of thin outer layer of connective tissue, and thick layer of high columnar epithelium, with cells having long necks and basal nuclei. Ducts of primary salivary glands (Figure 6, **sd**) thin, not passing through nerve ring, forming a loop, entering proboscis base parallel to esophagus. Ducts entering buccal mass posterior to ducts of accessory salivary gland.

Stomach long, narrow, situated beneath kidney and digestive gland, spanning one whorl. Stomach imperfectly preserved, transversal folds on its walls could not be clearly recognized.

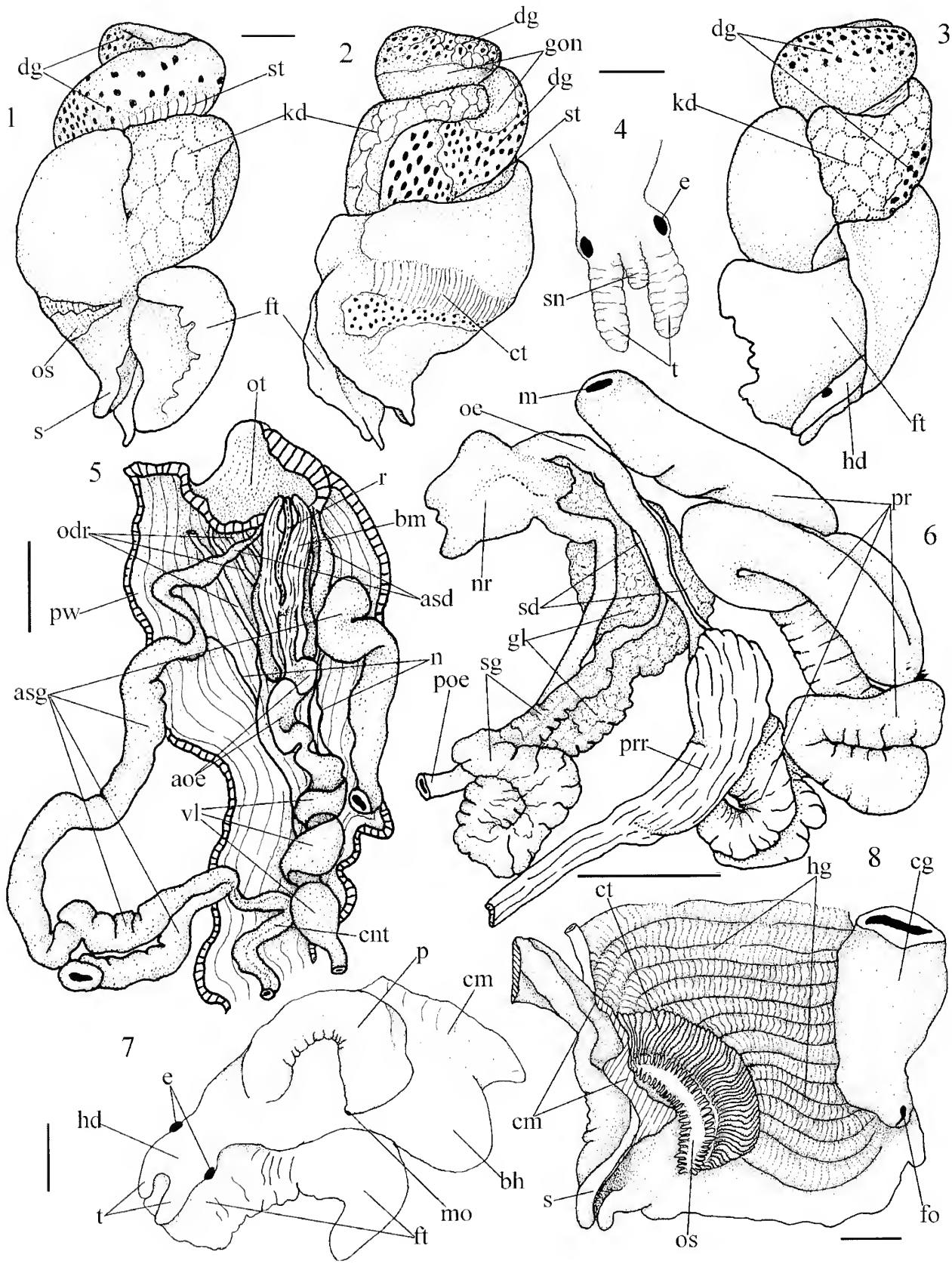
DNA Analysis: A total of 23 sequences were obtained for each of the two genes (including the outgroup *Cypraea cervinetta*). The sequences in the trimmed alignment were 521–541 bp for 12S and 489–679 bp for 16S. A χ^2 test of base homogeneity, uncorrected for phylogeny, indicated that base composition at each partition was not significantly different across all sites (16S: P=1.000; 12S: P=0.999).

Mutational saturation plots (results not shown) displayed evidence of saturation for both 12S and 16S sequences at the level of the ingroup-outgroup comparisons.

A partition homogeneity test performed in PAUP* (Swofford, 2000) did not reveal significant incongruence between the 16S and 12S datasets (P value=0.65).

The combined aligned dataset comprised 1300 nucleotide positions (12S: 581; 16S: 719), with the alignment of 301 positions considered uncertain, and thus excluded from subsequent analysis. Of the 999 included positions 536 were constant, 136 variable positions were parsimony-uninformative and 327 variable positions were parsimony-informative.

The MP analyses of each partition and of the combined dataset, produced topologies with very few nodes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supported by bs>50% (Figure 22). In all MP trees, the Rachiglossa, the Toxoglossa, the Muricidae, and the Buccinidae emerged as polyphyletic. In the analysis of the combined dataset, *Tritonoharpa+Plesiotriton* and the *Cancellaria* spp. comprised a nematoglossan clade, sister to the Olividae. Only seven nodes received a bootstrap support >90%.

Model test 3.7 selected by AIC the following models of nucleotide evolution: the TrN+I+G for 12S rDNA only and the TVM+I+G (transversional model) for 16S rDNA only. These models were adopted for ML analysis. MrModelTest2.2 selected by AIC the GTR+I+G substitution model both for 16S rDNA and for 12S rDNA; this model was used in the Bayesian analysis.

In the ML topology obtained for the concatened dataset (Figure 23), a sister-group relationship between *Tritonoharpa* and *Plesiotriton* was strongly supported (bs=99 and BPP=1). The Plesiotritoninae emerged as the sister group of the other Cancellariidae included in our analysis (*C. cooperi* and *C. cancellata*), albeit without strong support (bs=50 and BPP=0.89); the clade comprising all the nematoglossans (Cancellarioidea) was the sister-group of the remaining neogastropods (rachiglossans and toxoglossans). Toxoglossans (Conoidea) emerged as polyphyletic and basal to the stenoglossans. Within the rachiglossate group, a clade Olividae was basal (bs=95; not recovered in bayesian analysis), followed by a 'volutoid' clade (bs=95 and BPP=0.99), comprising Volutomitridae (*Microvoluta* sp.) and Costellariidae (*Vexillum* sp.) plus Ptychatractidae (*Latiromitra* sp). A clade formed exclusively of Muricidae (bs=92 and BPP=0.97) was the sister taxon to a clade of consisting of the 'buccinoid' families Nassariidae, Buccinidae, and Melongenidae) (bs=95 and BPP=0.95).

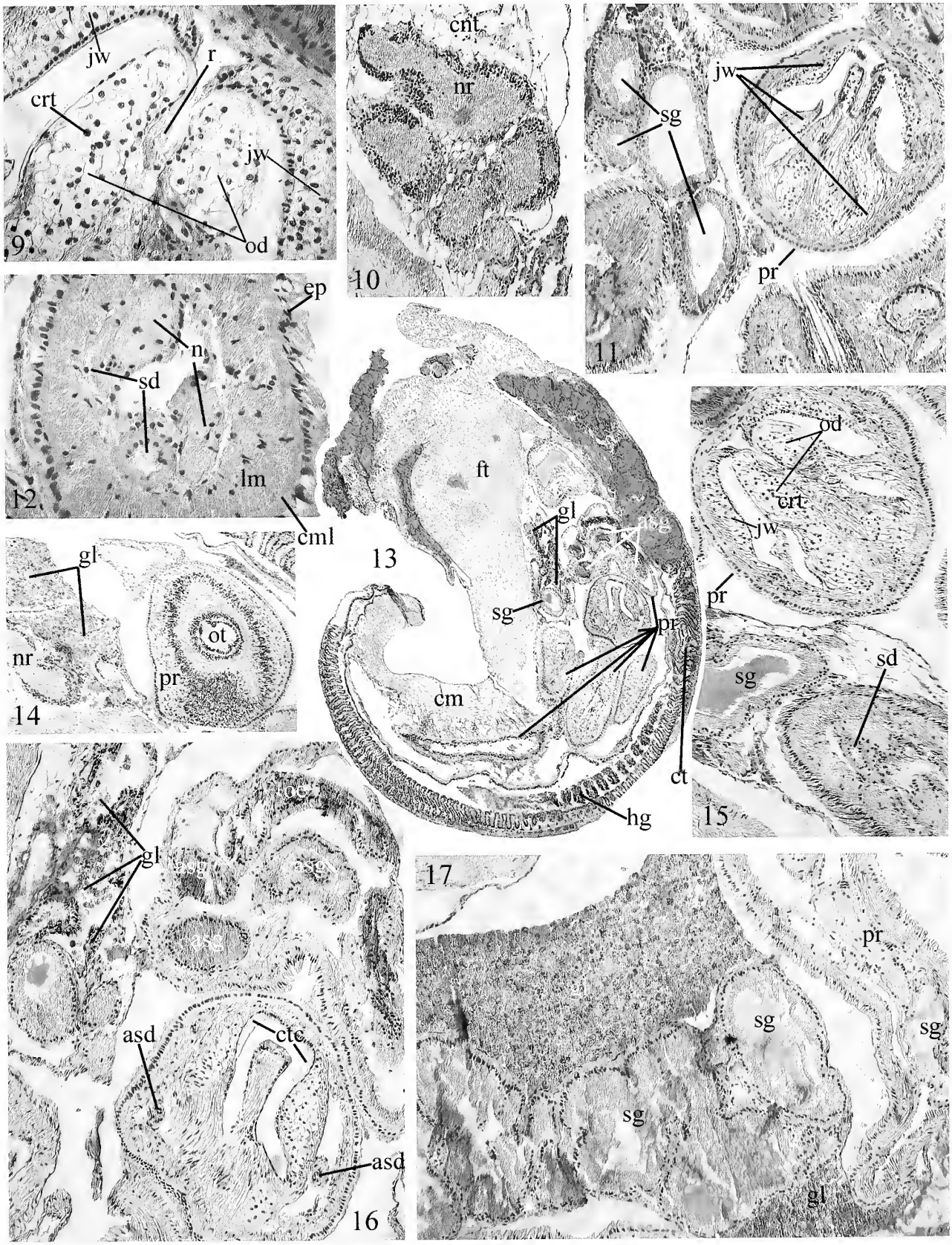
DISCUSSION

Morphology and Anatomy: Although *Tritonoharpa* is similar to the Colubrariidae and other neogastropods in the morphology of its head-foot, pallial complex, reproductive and excretory systems, and extremely long, coiled proboscis, it differs in its foregut anatomy. Beu and Maxwell (1987: 7) reported the lack of a radula in *T. antiquata* based on the examination of two specimens (one result admittedly "inconclusive", due to the extreme fragmentation of the specimen). We have observed the presence of a radula in at least three specimens. It is possible that Beu and Maxwell did not recognize a radula due to its extremely reduced size (<200 µm long). In some cancellariid species the radula may be present or absent (at different stages), as Oliver (1982) reported a radula only in the largest of two specimens of *Nothoadmete tumida* Oliver, 1982. The radula of *Tritonoharpa* has the typical nematoglossan structure, and is very similar to those of *Plesiotriton vivus* Habe and Okutani, 1981, and *Africotriton crebriliratus* (G. B. Sowerby III, 1903) (Beu and Maxwell, 1987, pls. 1 a–f and 13 a–d, respectively), comprising a single row of long, narrow, ribbon-like teeth. The peculiar tubular jaw surrounding the odontophore is typical of all Cancellariidae examined so far (Oliver, 1982; Harasewych and Petit, 1984, 1986; Simone and Birman, 2006) and may represent a synapomorphy of the Nematoglossa. Conceivably, the modification and reduction of the nematoglossan radula prompted the formation of protective jaws (**jw** in Figures 9, 15) around the median part of the odontophore (Figure 9, 15, **od**). This innovation was possibly induced by the necessity to either (1) raise the thin and long radular teeth, improving operational efficiency, and/or (2) strengthen the tip of the proboscis, which may be useful for suctorial feedng.

Tritonoharpa antiquata has two pairs of salivary glands. The accessory salivary glands have the typical tubular structure and location as described for other cancellariids (Graham, 1966; Harasewych and Petit, 1982, 1984, 1986). The primary salivary glands are tubular and located in the body haemocoel rather than in the proboscis. Such a position is unusual in cancellariids: it may be explained by the large size of these glands in *Tritonoharpa*, or alternatively it may be a plesiomorphic feature of the neogastropods.

Tritonoharpa antiquata has a large and well developed midgut gland located posterior to the nerve ring, which strongly resembles the gland of Leiblein of other neogastropods in its form and coloration. Although we have not detected any real duct connectig the gland to the esophagus, the only possible connection can be where the tissue of the gland and the esophagus are in contact, i.e. in the anterior portion of the gland, still posterior to the nerve ring. The tissue of this gland appears less structured than in the gland of Leiblein of other neogastropods (e.g., *Nucella lapillus*, Andrews and Thorogood, 2005; A. Richter, personal communication), although it is known that the general appereance of the gland can be related to feeding habits and the physiolog-

Figures 1–8. Anatomy of *Tritonoharpa antiquata*, Santo Is. (Vanuatu) and Aliguay Is. (Philippines). **1–3.** External view of the soft body of a female (BAU00303, Vanuatu). **4.** Head of a female (BAU00269, Philippines). **5.** Anterior section of the proboscis of a female (BAU00301, Vanuatu), dissected dorsally. **6.** Foregut anatomy of a female (BAU00268, Philippines). **7.** Head-foot of a male (BAU00269, Philippines). **8.** Mantle of a female (BAU00268, Philippines). Scale bar – 1 mm. Abbreviations: **aoe,** anterior esophagus; **asd,** accessory salivary duct; **asg,** accessory salivary gland; **bh,** body haemocoel; **bm,** buccal mass; **cg,** capsule gland; **cm,** columellar muscle; **cnt,** connective tissue; **ct,** ctenidium; **dg,** digestive gland; **e,** eye; **fo,** female orifice; **ft,** foot; **gl,** gland of Leiblein; **gon,** gonad; **hd,** head; **hg,** hypobranchial gland; **kd,** kidney; **m,** mouth; **mo,** male orifice; **n,** nerves; **nr,** nerve ring; **odr,** odontophoral retractors; **oe,** esophagus; **os,** osphradium; **ot,** oral tube; **p,** penis; **poe,** posterior esophagus; **pr,** proboscis; **prr,** proboscis retractors; **pw,** proboscis wall; **r,** radula; **s,** siphon; **sd,** salivary duct; **sg,** salivary gland; **sn,** snout; **st,** stomach; **t,** tentacles; **vl,** valve of Leiblein.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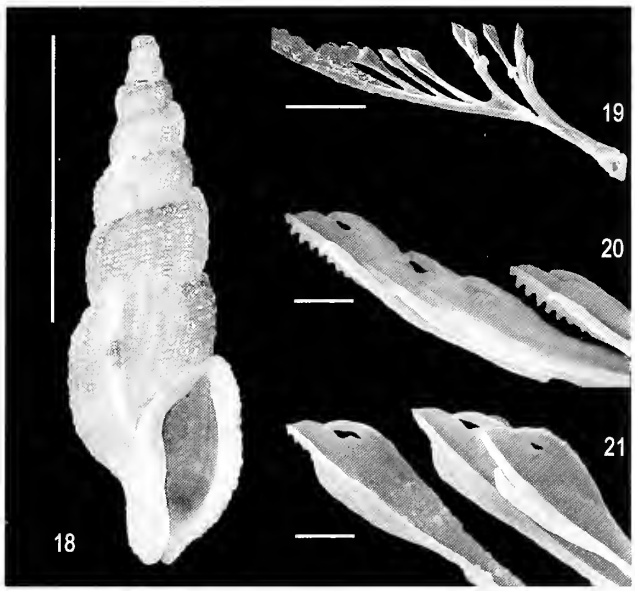

Figures 18–21: Shell and radula of *Tritonoharpa antiquata*. **18.** Shell, off Tayud Is., Lilo-an (Cebu, Philippines) (photo courtesy, G. and P. Poppe). **19–21.** Radula, Mactan (Philippines; BAU00269). Scale bars: 10 mm (18), 50 μm (19), 5 μm (20–21).

ical state of the specimens (Andrews and Thorogood, 2005; A. Riehter, personal eommunieation). Large globular cells of this gland, with large nuclei and multiple nucleoli and granules in the cytoplasm indicate high seeretion activity; the presence of vesieles filled with granules suggests an apocrine seeretion mechanism. While the diet of *Tritonoharpa antiquata* is unknown, it is likely that individuals in this species are suctorial, feeding on body fluids as do other cancellarioideans. This eonjecture is supported by the extreme modification of the radula, which suggests use for piereing rather than rasping (Oliver, 1982; Petit and Harasewych, 1986), by the tubular nature of the jaw, and by the large stomaeh resembling that of the haematophagous Colubrariidae (Ponder, 1968; Oliverio and Modiea, in press). Furthermore, haematophagy has been already reported for the caneellariine *Cancellaria cooperi* Gabb, 1865 (O'Sullivan et al., 1987), while other caneellariid species have been observed feeding on bivalves (*Trigonostoma scalariformis* (Lamarck, 1822)), sand-dwelling gastropods (*Trigonostoma scalata* (Sowerby, 1832)) and, in aquarium, on fish pieces and squid eggs (Loch, 1987).

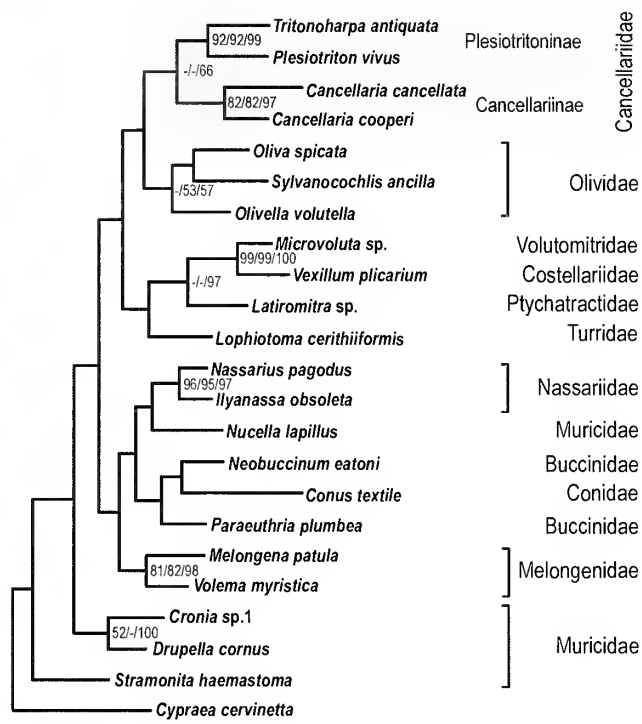

Figure 22: Maximum Parsimony topology obtained for the combined molecular dataset. Numbers at nodes represent Bootstrap values (1000 replicates) in the anlysis of the 12S, 16S, and combined datasets, respectively.

During several days of aquarium observations (Santo 2006 expedition: MO, unpublished), two specimens of *T. antiquata* did not show any feeding activity in the presence of living specimens of various species of fishes.

The peeuliar long and spirally convoluted valve of Leiblein, which differs from the pyriform valve of other Neogastropoda, has been also reported in *Plesiotriton vivus* (Kantor and Fedosov, 2009). Its functional significance deserves further investigation.

Phylogeny: The MP analyses of each partition and of the combined dataset, produced highly implausible results, particularly as the Rachiglossa, the Muricidae and the Buccinidae all emerged as polyphyletic (Figure 22), yet with a very few nodes with strong bootstrap support. This was probably due to the inclusion in our dataset of some highly divergent sequences (e.g., *Stramonita haemastoma* (Linnaeus, 1767), and *Conus textile* Linnaeus, 1758), a

Figures 9–17. Histology of *Tritonoharpa antiquata*, Santo Is. (Vanuatu; BAU00302, female. **9.** Cross-section of odontophore and radula. **10.** Nerve ring. **11.** Anterior part of the proboscis with buccal mass and salivary glands, stained with hematoxylin and eosin. **12.** Cross-seetion through the posterior part of the proboscis with primary salivary ducts and nerves. **13.** General view of the cross-section through the medial region of the last whorl of the animal. **14.** Cross-section of the proboscis at the level of the oral tube and medial part of the midgut gland. **15.** Anterior part of the proboscis with buceal mass and salivary glands, stained with alcian blue. **16.** Cross-seetion of the proboscis with accessory salivary glands and their ducts. **17.** Longitudinal section through the posterior parts of the midgut gland and salivary glands. Abbreviations: **asd,** aeeessory salivary duct; **asg,** accessory salivary gland; **cm,** eolumellar muscle; **cml,** eireular muscles; **cnt,** connective tissue; **crt,** odontophoral cartilages; **ct,** ctenidium; **etc,** cuticle; **ep,** epithelinm; **ft,** foot; **gl,** gland of Leiblein; **hg,** hypobranchial gland; **lm,** longitudinal muscles; **lw,** lateral wings of the odontophoral cartilage; **modr,** middle part of the odontophoral eartilage; **n,** nerves; **nr,** nerve ring; **oc,** esophagus; **ot,** oral tube; **pr,** proboscis; **r,** radula; **sd,** salivary duct; **sg,** salivary gland.

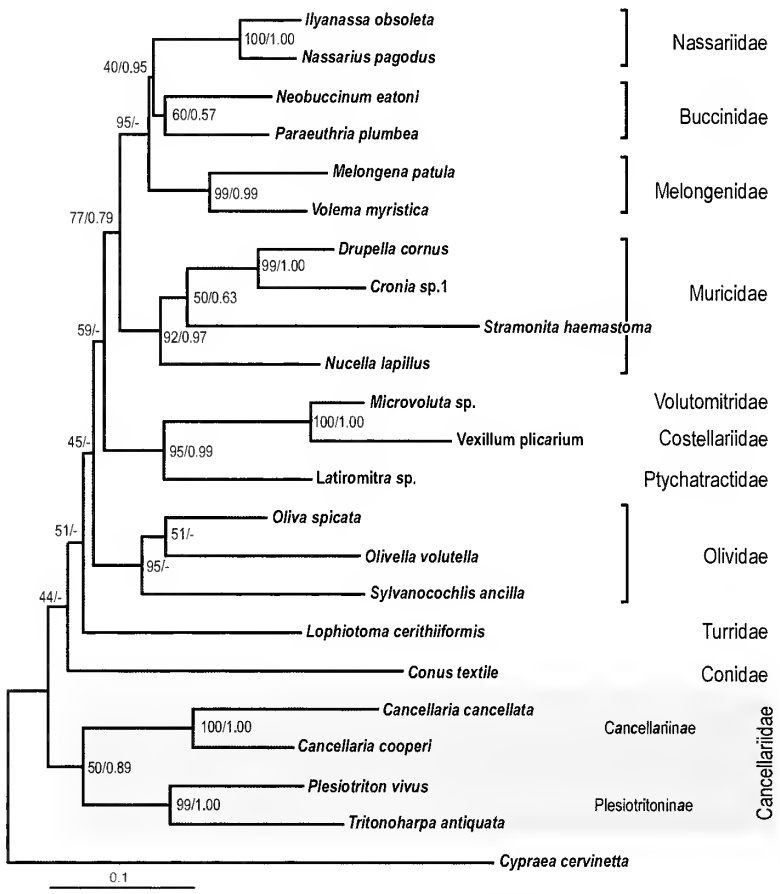

Figure 23: Partitioned Maximum Likelihood topology obtained for the molecular dataset. Numbers at nodes represent Bootstrap values/Bayesian Posterior Probability.

situation in which MP is expected to perform poorly (Felsenstein, 1978; Kim, 1996; Holder and Lewis, 2003). Therefore, MP results will not be deseribed and diseussed in details.

The ML and BI phylogenetie analyses of the moleeular datasets confirms Beu and Maxwell's plaeement of *Tritonoharpa* in the Cancellariidae within a plesiotritonine group. It also suggests that eaneellariids eould be the sister-group to other neogastropods, in agreement with neogastropod phylogenetic hypotheses based on anatomieal characters (Kantor, 1996, 2002; Strong, 2003) and larger molecular datasets (Oliverio and Modiea, in press).

The presence of a midgut gland resembling (and possibly homologous to) the neogastropod gland of Leiblein in *Tritonoharpa* raises some interesting questions on the evolution of the foregut. In fact, current hypotheses interpret the lack of separation between the midgut gland and esophagus in the cancellariids as indicating that the elongation site is the mid-esophagus. In the rachiglossans the elongation site is the anterior esophagus, causing the detachment of the glandular tissue from the oesophageal walls and the formation of the gland of Leiblein (Ponder, 1974). If further studies on the midgut gland of the Plesiotritoninae (e.g., biochemical characterization of the seeretion, exaet loealization of the eonneetion to the esophagus) will eonfirm its homology with the neogastropod gland of Leiblein, the possession of a separate gland should be eonsidered as an apomorphy of the Neogastropoda (instead of only of raehiglossans + toxoglossans). It may thus not be the site of elongation of the esophagus that determined the formation of the gland of Leiblein. The presenee of glandular band of tissue, and not a separate gland, in other eaneellariids (Harasewyeh and Petit, 1982; 1984; 1986) eould be eonsidered as a seeondary reduetion. Alternatively, either the plesiotritonine midgut gland or the separate glandular tissue of other eaneellariids may not be homologous to the true gland of Leiblein. The development of a eompensatory glandular region, has already been reported for other neogastropods, where it is assoeiated with a reduced or absent gland of Leblein (e.g., the glandular mid-posterior esophagus of Colubraridae: Ponder, 1968, 1973; Oliverio and Modiea, in press).

The buceal mass is displaeed posteriorly from the proboscis tip of eancellarioideans by the length of the oral tube. This condition does not correspond to a basal position (as in the toxoglossans), whieh has been hypothesized as the plesiomorphie state for the aneestral neogastropod

(Kantor, 1996; 2002). An intermediate and variable condition in the buccal mass position is observed in olivids (Kantor, 1996; 2002), which our ML tree shows to be a basal clade within the rachiglossan radiation (Figure 23).

In our phylogeny, several clades are well supported (Figure 23). In a 'volutoid' clade, comprising *Latiromitra*, *Vexillum*, and *Microvoluta* (members of Ptychatractidae, Costellariidae, and Volutomitridae respectively), at least the first two species exhibit a primitive arrangement of the foregut (Bouchet and Kantor, 2000; Ponder, 1972). Ptychatractids have been recently treated as a separate family (Bouchet and Rocroi, 2005), but they had been included as a subfamily of the Turbinellidae (e.g., Bouchet and Warén, 1985), which are a group displaying remarkable variation among the recognized subfamilies (Ponder, 1974; Kantor and Bouchet, 1997). The placement of *Latiromitra* in our analysis suggests that a 'volutoid' affinity of the ptychatractids may exist, as suggested by, e.g., Thiele (1929) or Cernohorsky (1970). A 'buccinoid' clade is recognizable in a more derived position (including members of the families Nassariidae, Buccinidae, and Melongenidae), sister to a clade constituted exclusively by Muricidae. This result is in agreement with a recent morphology-based phylogenetic hypothesis (Strong, 2003).

It is evident that cancellariids are a key group for understanding neogastropod evolution, although their anatomical disparity is still largely unexplored. As more anatomical data on *Plesiotriton* and other cancellariids become available, a new light could be shed on the evolution of the foregut in Neogastropoda and on the early radiation of the group.

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The genus *Olivella* Swainson, 1831(Gastropoda: Olividae) in Argentine waters

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ABSTRACT

The genus *Olivella* is represented in Argentine waters by four species: *Olivella puelcha*, *O. tehuelcha*, *O. orejasmirandai*, and *O. santacruzence*. These species are redescribed, and type material, radulae, opercula, and penes are illustrated by SEM images. The geographic distribution of each species is provided based on field observations as well as on museum reeords. A synonymy for each speeies is presented.

Additional keywords: Argentina, Patagonia, Neogastropoda, *Olivina*

INTRODUCTION

The family Olividae is very well represented in the southwestern Atlantic. Rios (2009) recorded 35 species living in Brazilian waters. Among the genera belonging in this family, *Olivella* is probably the most specious, particularly in Brazil, where Rios (op. cit.) documented the occurrence of 20 species.

Among previous papers describing species of *Olivella* from southwestern Atlantic waters, those of Klappenbach (1962, 1964, 1986, 1991a, 1991b, 1991c) established the basics for the study of the taxonomy of this genus in Argentina, Uruguay, and Brazil. In addition to these papers, Castellanos and Fernández (1965) described the southernmost record for the genus: *Olivella santacruzence*, a species known only from dead shells.

Most of the early literature on South American *Olivella* described the shells and only rarely the radulae (which had never been illustrated through SEM), but not anatomical features such as penes. None of the papers published by those early authors reviewed the type material of the oldest species.

Recently, Absalão (2000) and Absalão and Pimenta (2003) started a series of new studies of the genus *Olivella* with emphasis on the Brazilian fauna. The present paper supplements these studies by including redescriptions, corroborating ranges, and including SEM illustrations of shells, radulae, penes and, when available, egg capsules for all known Argentine species.

MATERIALS AND METHODS

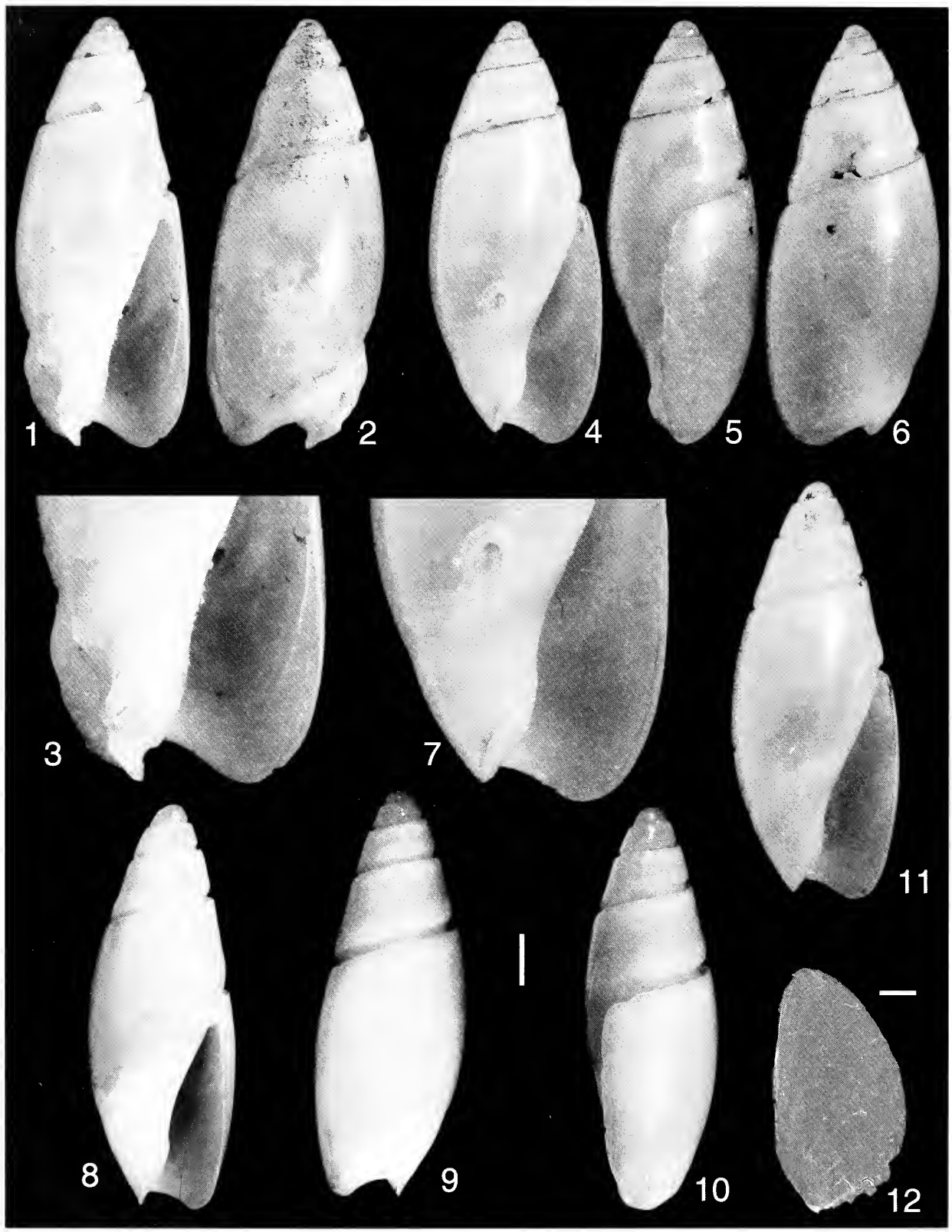
This study is based on material in the collections of the following institutions: Museo Argentino de Ciencias Naturales, Buenos Aires (MACN); Museo de La Plata (MLP) and Museo Nacional de Historia Natural de Montevideo (MNHNM). Type material from the Natural History Museum (BMNH), London, and the Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil, was also studied.

Live specimens were collected on the sandy infralittoral zone of the following localities from Chubut province: Punta Villarino on the Golfo San José (42°24′ S, 64°15′ W) on December 2002, 2003, 2004, 2007, and March 2005, in about 1–3 m depth during low tide, Punta Pardelas (42°37′ S, 64°15′ W) and off Estancia El Pedral, Golfo Nuevo (42°56′ S, 64°25′ W). All localities are along the perimeter of the Valdés Peninsula, Chubut Province, Argentina. Some of the specimens were frozen to allow for observation of soft tissues in an expanded condition. Radulae were cleaned with Clorox [NaClO] and sonicated in an ultrasonic cleaner, mounted, coated with gold, and photographed using a Philips XL30 scanning electron microscope at MACN. All shells where photographed using a Nikon D100 camera and digitally processed with the appropriate software.

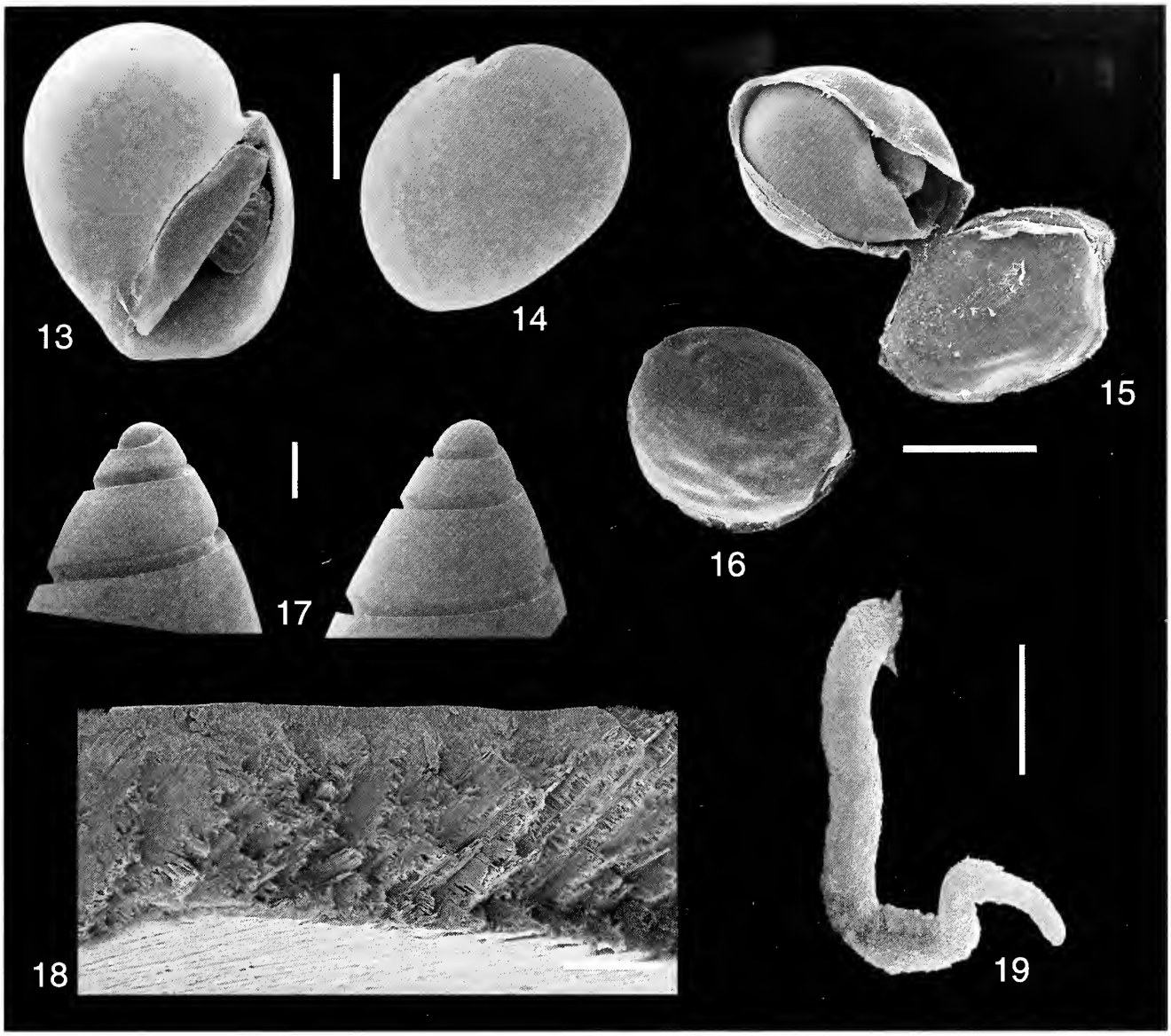
SYSTEMATICS

Class Gastropoda Cuvier, 1791
Subclass Orthogastropoda Ponder and Lindberg, 1995
Order Sorbeoconcha Ponder and Lindberg, 1995
Infraorder Neogastropoda Thiele, 1929
Family Olividae Latreille, 1825

Genus *Olivella* Swainson, 1831



Figures 1–12. *Olivella puelcha* (Duclos, 1835). **1–3.** Syntype of a female of *Olivina telnelchana* d'Orbigny, BMNH 1854.12.4.409, Bahía San Blas, Argentina. **4–7.** Syntype (male) of *Olivina telnelchana* d'Orbigny, BMNH 1854.12.4.409, Bahía San Blas, Argentina. **8–10.** MACN-In 16828-1, coast of Buenos Aires Province. **11.** Syntype (male) of *Olivina telnelchana* d'Orbigny, BMNH 1854.12.4.409. **12.** External view of the operculum. Scale bars: All shells = 1 mm; Figure 12 = 400 µm.



Figures 13–19. *Olivella puelcha* (Duclos, 1835). **13.** Apertural view of an embryonic shell. **14.** Adapertural view of another embryonic shell. **15.** Open egg capsule with an embryo inside. **16.** Closed egg capsule. **17.** Detail of the protoconch, scale bar = 500 µm. **18.** Shell ultrastructure, fraeture surface commarginal. **19.** Penis, critical-point dried. Scale bars: Figures 15, 16 = 500 µm; 18 = 50 µm; 19 = 1000 µm.

Type Species: *Oliva dama* Mawe, 1828 by subsequent designation (Dall, 1909).

Olivella puelcha (Duclos, 1835)
(Figures 1–19, 39–41)

Oliva puelcha Duclos, 1835: pl. 4 bis, fig. 1–6, 20.
Oliva tehueleliana d'Orbigny, 1839: pl. 59, fig. 7–12; Marrat, 1871: 38, fig. 457.
Olivina tehueleliana d'Orbigny, 1840: 418.
Oliva tehuelcha Duclos in Chenu, 1844: 6; Chenu, 1845: pl. 5, fig. 1–6.
Olivancillaria aurieularia plata Ihering, 1908: 432.
Olivella tehueleliana (d'Orbigny, 1841).—Carcelles, 1944: 258; Castellanos and Fernández, 1965: 103, fig. 6–9; Castellanos, 1970: 122 pl. 10, fig. 5.
Olivella tehueleha (Duclos, 1840).—Rios, 1985: 114, fig. 506; 1994: 144, fig. 630; 2009, fig. 687 (description is *O. puelcha*).
Olivella puelcha (Duclos, 1835).—Klappenbach, 1991b: 121.
Olivella plata Ihering, 1909 [sic].—Castellanos and Fernández, 1965: 101, fig. 4–5, 12, 13; Castellanos, 1970: 123; Rios, 1985: 113, fig. 504; 1994: 145, fig. 628; Borzone, 1995: 52, figs. 28, 29; Rios, 2009: 275.
Olivella plata (Ihering, 1908).—Pastorino, 1995: 10, Pl. 2, fig. 12; Pastorino, 2007: 1, Fig. 1, A–J.

Description: Shell of small size for genus, (to 11 mm), subquadrate, of 5 completely smooth, flat whorls. Protoconch of about 1.5–2 smooth whorls (Figure 17). Transition to teleoconch indistinct. Spire of medium size, suture channeled, narrowly open. Parietal callus smooth, broad, thick; Columella with one plait weakly divided in middle, becoming two plaits toward aperture. Fasciolar band moderately wide, posterior groove weak. Sexual dimorphism evident in shells of this species: Shells of females have wide vertical anterior groove, adjacent to parietal callus and columellar pillar structure. The groove curves adaxially at tip of also adaxially curved pillar, is absent in shells of males where parietal callus, apparently "fills in" groove described for female shells. Color always bright-white. Shell ultrastructure composed of thick outer layer of crossed-lamellar crystals and an extremely thin inner layer of apparently amorphous constitution. Radula rachiglossate, rachidian teeth wide, slightly concave, with convex, somewhat elliptical base. Cusp flat, rectangular, with rounded tips near center of tooth, becoming smaller, more broadly spaced, and sharper toward sides of tooth. Lateral teeth, flat, smooth, curved, blunted at ends, with thin, polygonal attachment area at base. Operculum semicircular, filling the whole aperture; nucleus subterminal, somewhat lateral. Growth lines, closely spaced over entire surface (Figure 12). Penis very long, thin, tapering, with curved tip at distal end (Figure 19). Egg capsules semicircular, each containing a single embryo (Figures 13–16).

Type Material: Thirteen syntypes of *Olivina tehuelehana* d'Orbigny, BMNH 1854.12.4.409, two females and 11 males. The type material of *Olivaneillaria auricularia plata* Ihering was not localized, apparently it was never deposited.

Type Locality: "Côtes sablonneuses des îles de la baie San-Blas", sandy coasts of San Blas Bay Islands for *O. tehuelchana*; Punta Piedras, Buenos Aires Province, for *Olivella plata* Ihering.

Other Material Examined: MACN-In 30306, 37°28′ S, 56°20′ W; MACN-In 14348, 38°35′ S, 57°09′ W, in 102 m; MACN-In 16289, (all will hermit crabs), 38°52′ S, 56°20′ W in 90 m, Mar del Plata; MACN-In 16496-1; 16496, both from Punta Médanos; MACN-In 6619-42; 6619-41, Monte Hermoso, Buenos Aires province; MACN 16828-1, Buenos Aires province coast; MACN-In 30333, 16630, 30317, 30334, 20244, all from Bahía San Blas, Buenos Aires province; MACN-In 37603, Punta Villarino, Golfo San José, 42°24′ S, 64°15′ W, 1–3 m depth during low tide; MACN-In 37604, Punta Pardelas, 42°37′ S, 64°15′ W, Golfo Nuevo, Chubut, 6 m depth.

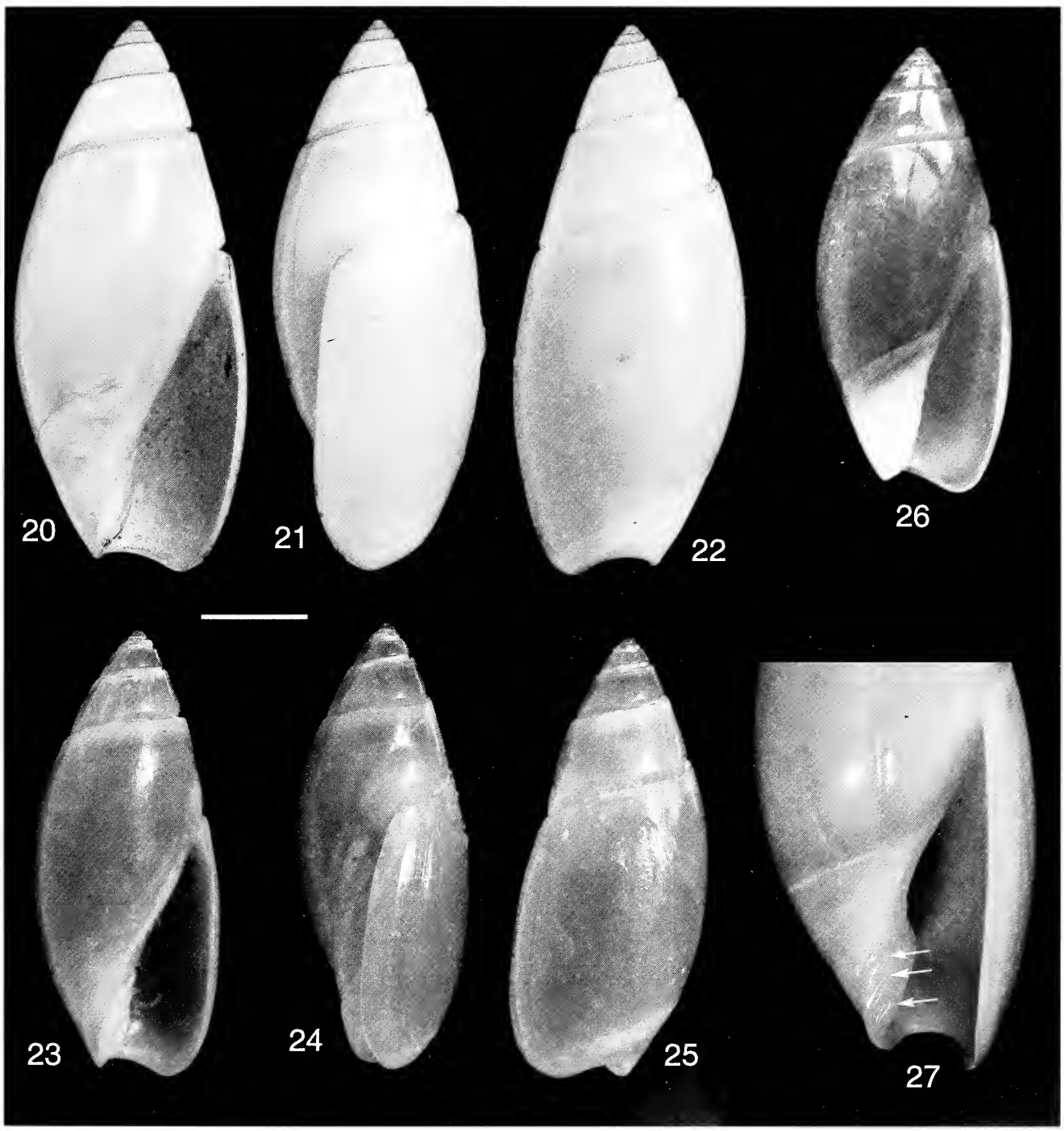
Geographic Distribution: Rio Grande do Sul, Brazil (Rios, 1994; 2009) to Puerto Pirámide, Golfo Nuevo, Chubut, Argentina.

Remarks: In a short note on the genus *Olivella*, Klappenbach (1991b) reviewed the taxonomic history of the two South Atlantic species of *Olivella* described by d'Orbigny. A number of factors led subsequent authors to confuse the true identity of these species and preventing correct placement of the material in the appropriate taxon. D'Orbigny collected his original material during his voyage to South America and, after returning to France, described them as *Oliva tehuelehana* and *Oliva puelchana*, publishing the plates in 1839 and the descriptions in 1840. However, before publication of either, he apparently sent his illustrations to Duclos. In 1835, Duclos published plates with illustrations of these two species in his monograph of the genus *Oliva*, apparently reproduced from the illustrations that d'Orbigny sent to him. Duclos changed the final part of the names ("*pueleha*" for "*puelehana*" and "*tehueleha*" for "*tehuelehana*") and also transposed the names in the plates. In fact, as d'Orbigny stated in his publication several years later (1840: 418, footnote), both names are transposed in Duclos's monograph, so Duclos's *Oliva pueleha* and *O. tehueleha* are d'Orbigny's *Olivina tehuelehana* and *O. puelehana*, respectively. As Duclos's monograph was published earlier, his names have priority over d'Orbigny's. Aguirre (1993: 30) was aware of Duclos' earlier names. However, she maintained d'Orbigny's names although she considered them synonyms [as *Olivella puelchana* (d'Orbigny, 1840)]. Unfortunately, she designated (Aguirre, 1993: 30, pl. I, fig. 5) the lectotype for *Olivina puelehana* d'Orbigny, 1840 [=*Olivella tehueleha* Duclos], a specimen from lot BMNH 1854.12.4.409, which is actually one of the 13 syntypes of *Olivina tehuelehana* d'Orbigny, 1840 [= *O. pueleha* Duclos]. Therefore, such lectotype designation is invalid under the provisions of Article 74.2 of the International Code of Zoological Nomenclature (ICZN, 1999). Aguirre illustrated the specimen she selected as lectotype, which is clearly a male specimen of *O. puelcha* (Duclos).

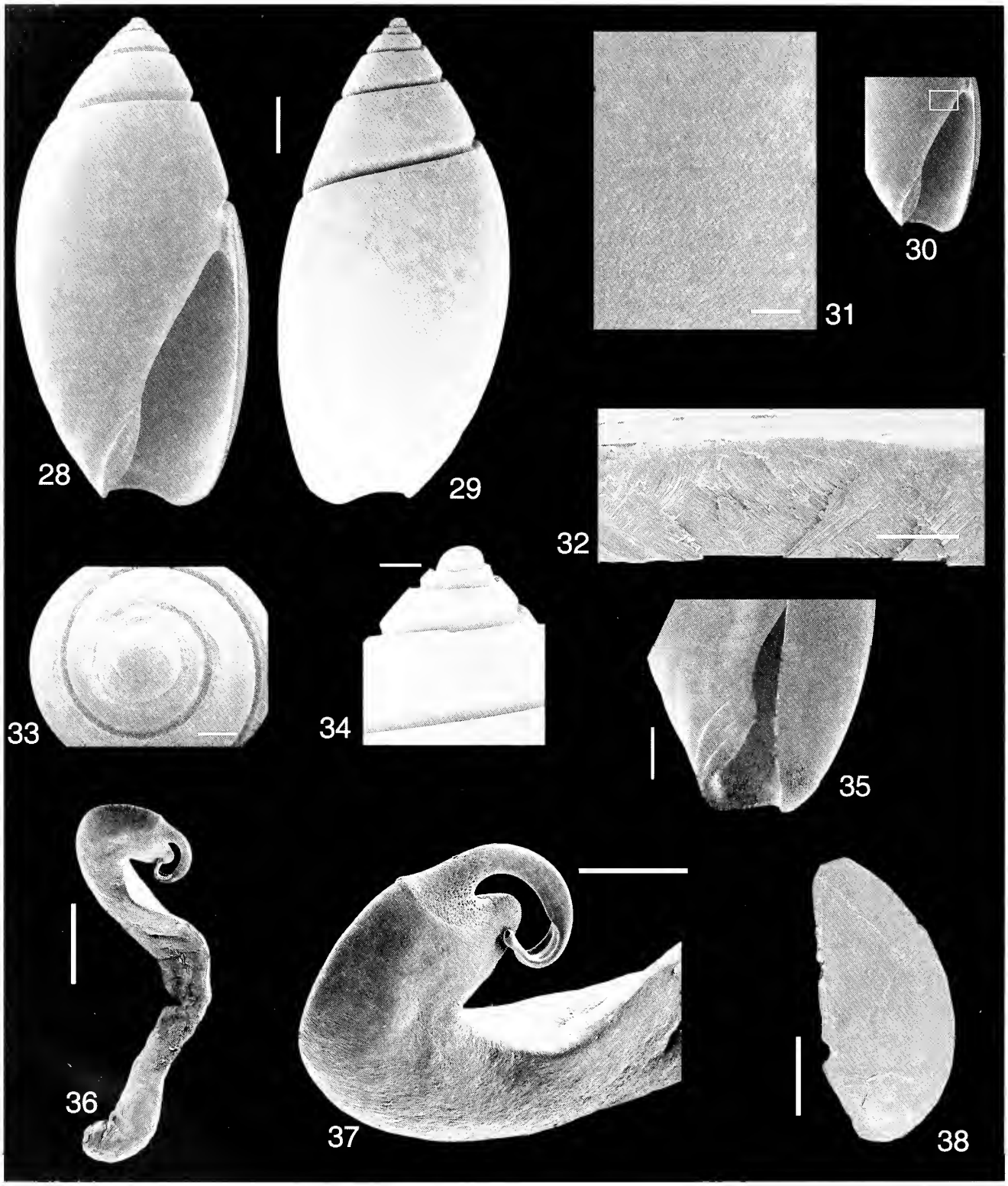
Olivella plata was described originally as a subspecies of *Olivancillaria auricularia* in a very short description together with other Quaternary species (Ihering, 1908). Ihering mentioned *Olivella tehuelehana* two lines above that, although it is difficult to believe that he could not differentiate between the genera *Olivaneillaria* and *Olivella*. Castellanos and Fernández (1965: 103) reported seeing the type specimen. The only material they had available was lot MACN-In 6619, which contained several specimens and included a handwritten label from Doello-Jurado (former curator of the Invertebrate Division at the MACN), explaining that the material was split from a larger lot (also housed at the MACN) and identified as "*O. tehuelchana* d'Orbigny" (=*O. pueleha* Duclos) by Ihering. These facts apparently lead Castellanos and Fernández to the erroneous conclusion that this was the type material of *O. plata*. The locality data for this lot was Monte Hermoso, while the type locality for *O. plata* is Punta Piedras (Ihering, 1908: 432). Part of Ihering's type material is housed at the MACN and part at the MZUSP. After a careful revision of both collections it is evident that the type material of *O. plata* was not deposited in either of these institutions and was never illustrated. Nevertheless, the characteristic shape

of the anterior part of the females of *O. puelcha* allows the identification with some confidence and the posterior synonymization of *O. plata*.

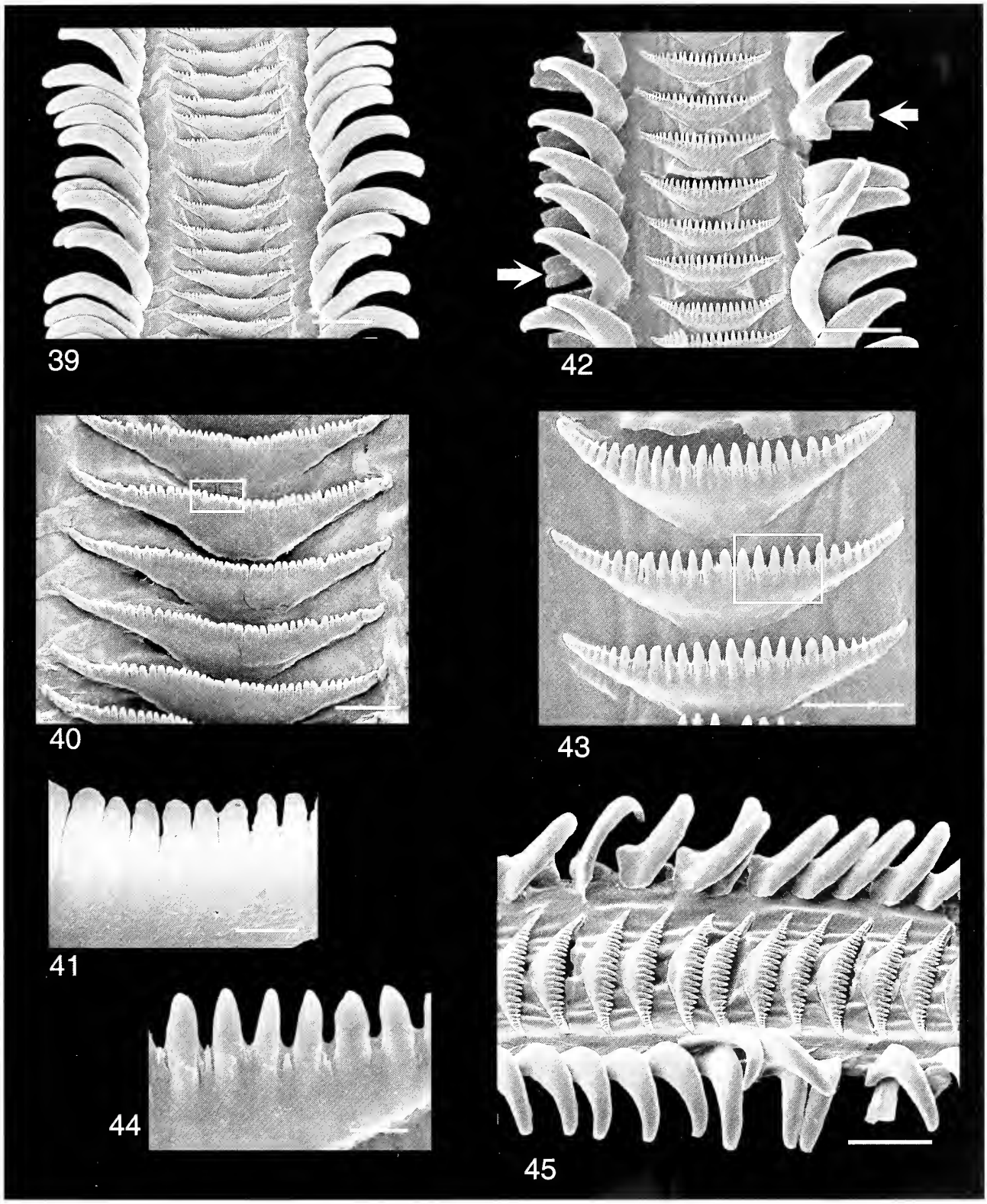
Pastorino (2007) described the sexual dimorphism of this species (as *O. plata*). A careful study of the entire type series of *Olivina tehuelchana* d'Orbigny [=*Olivella puelcha* Duclos] housed at the BMNH allows the recognition of both sexual morphs, establishing that the males were described by Duclos and d'Orbigny as *Oliva puelcha* and *Olivina tehuelchana*, respectively and, several



Figures 20–27. *Olivella tehuelcha* (Duclos, 1835). **20–22.** Syntype of *Olivina puelchana* d'Orbigny, BMNH 1854.12.4.408, Bahía San Blas, Argentina. **23–25.** Another syntype of *Olivina puelchana* d'Orbigny, BMNH 1854.12.4.408, Bahía San Blas. **26.** MACN-In 37605, Punta Pardelas, Golfo Nuevo, Argentina. **27.** Detail of the columella plaits of MACN-In 16675. Scale bar = 3 mm.



Figures 28–38. *Olivella tehuelcha* (Duclos, 1835). **28–29.** Two views of MACN-In 37605, Punta Pardelas, Golfo Nuevo, Argentina, coated for SEM. **30–31.** Detail of the parietal callus. **32.** Ultrastructure, fracture surface commarginal. **33–34.** Protoconch, **33.** apical view. **34.** Lateral view. **35.** Detail of columellar plaits of MACN-In 37605. **36–37.** Penis, critical-point dried. **37.** Detail of the papilla. **38.** SEM, external view of the operculum. Scale bars: Figures 30, 31 = 100 μm; 32 = 180 μm; 33 = 200 μm; 34 = 300 μm; 35 = 800 μm; 36 = 1000 μm; 37 = 500 μm; 38 = 1000 μm.



Figures 39–45. *Olivella* radulae. **39–41.** *Olivella puelcha* (Duclos, 1835). **39.** General view. **40.** Detail of the rachidian teeth of the radula in Figure 39. **41.** Detail of the cusps of the rachidian in Figure 40. **42–45.** *Olivella tehuelcha* (Duclos, 1835). **42.** General view, arrows head quadrangular piece underlying lateral teeth. **43.** Detail of the rachidian teeth of the radula in Figure 42. **44.** Detail of the cusps of the rachidian in Figure 43. **45.** Lateral view of the radula. Scale bars: Figure 39 = 50 µm; 40 = 20 µm; 41 = 5 µm; 42 = 100 µm; 43 = 20 µm; 44 = 10 µm; 45 = 100 µm.

deeades later, the females as *Olivancillaria auricularia plata* by Ihering.

Borzone (1995) briefly described the egg eapsules from material colleeted in southern Brazil (as *O. plata*). The embryos and egg capsules, illustrated here (Figures 13–16), were collected during the southern hemisphere summer (November–January).

Olivella tehuelcha (Duelos, 1835)
(Figures 20–38, 42–45)

Oliva tehuelcha Duclos, 1835: pl. 4 bis, fig. 7–14, 21.
Oliva puelchana d'Orbigny, 1839: pl. 59, fig. 13–19; Marrat, 1871: 35, figs. 461, 462.
Olivina puelchana d'Orbigny, 1840: 418.
Oliva puelchana Duclos in Chenu, 1844: 6.
Oliva puelcha Duclos in Chenu, 1845: pl. 5, figs. 7–14.
Olivella jaspidea Gmelin.—Dall, 1890: 310 (according to Klappenbach, 1991c).
Olivella puelchana d'Orbigny.—Formica-Corsi, 1900: 80, fig. 19; Carcelles, 1944: 159; Castellanos and Fernández, 1965: 103, figs. 1–3; Castellanos, 1970: 122, pl. 10, fig. 6; Aguirre, 1993: 30, pl. I, fig. 5.
Olivella tehuelcha (Duclos, 1835).—Klappenbach, 1964: fig. 5; 1991b: 121; Abbott and Dance, 1986: 194.
Olivella puelcha (Duclos, 1840).—Rios, 1985: 114, fig. 505; Calvo, 1987: 164, fig. 151; Rios, 1994: 144, fig. 630.

Description: Shell medium size for the genus, up to 15 mm in length, subovate, elliptie, solid, with five smooth, flat whorls. Protoconch with at least two whorls, totally smooth; transition to teleoeoneh not elearly defined (Figures 33, 34). Color variable, with light or dark brownish baekground, some specimens with brighter, elosely arranged, flamules. Spire elevated, <0.5 total length; suture ehanneled, very deep; parietal callus with very weak, microseopic, regularly arranged pustules (Figure 31). Columella with only two plaits, with obsolete intermediate plait oecasionally present and visible towards interior of aperture (Figures 27, 35, arrows). Faseiolar band thin, whitish, posterior groove distinet; anterior portion of faseiolar band dark. Shell ultrastrueture eomposed of single layer of erossed-lamellar strueture (Fig. 32). Radula rachiglossate (Figures 42–45), with 28–30 rows of teeth. Rachidian teeth eliptieal with regularly curved base; 23–26 denticles of same size along mid-seetion, but abruptly diminishing to the sides. Smaller, almost obsolete denticles always present. Lateral teeth, typieally curved, with sharp end and flat profile. A quadrangular, flat pieee, is always present under lateral teeth (Figure 42, arrow head).

Operculum extremely thin, translueid, yellowish, elliptical, with subterminal nueleus. Growth lines eover entire operculum surface (Figure 38).

Penis large and flat, ending in long and curved papilla when protruded. Tip of the penial papilla flat and tapering (Figures 36, 37).

Type Material: Sixteen syntypes of *Olivina puelchana*, BMNH 1854.12.4.408. Color variation is evident in the type series, ranging from dirty white and yellowish, to dark brown. Two syntypes are illustrated here in Figures 20–22 and 23–25.

Type Locality: "Baie de San Blas", south of Buenos Aires Provinee, Argentina.

Other Material Examined: MACN-In 16675, Mar del Plata, males and females; MACN-In 9174, Golfo San José, several speeimens; MACN-In 19670 all dead, oeeupied by sipunculids, Isla Trinidad, Bahía Blanea; MACN-In 8889 two shells with hermit crabs; MACN-In 30331, Mar del Plata, 32–36 m, two speeimens, several shells; MACN-In 30318, Mar del Plata, all speeimens; MACN-In 14348, 38°35′ S, 57°09′ W, in 100 m, 1 shell; MACN-In 24150, 36°24′ S, 55°53′ W, 1 specimen, 2 shells; MACN-In 20243, Bahía San Blas; MACN-In 14349, Mar del Plata, all with hermit crabs.

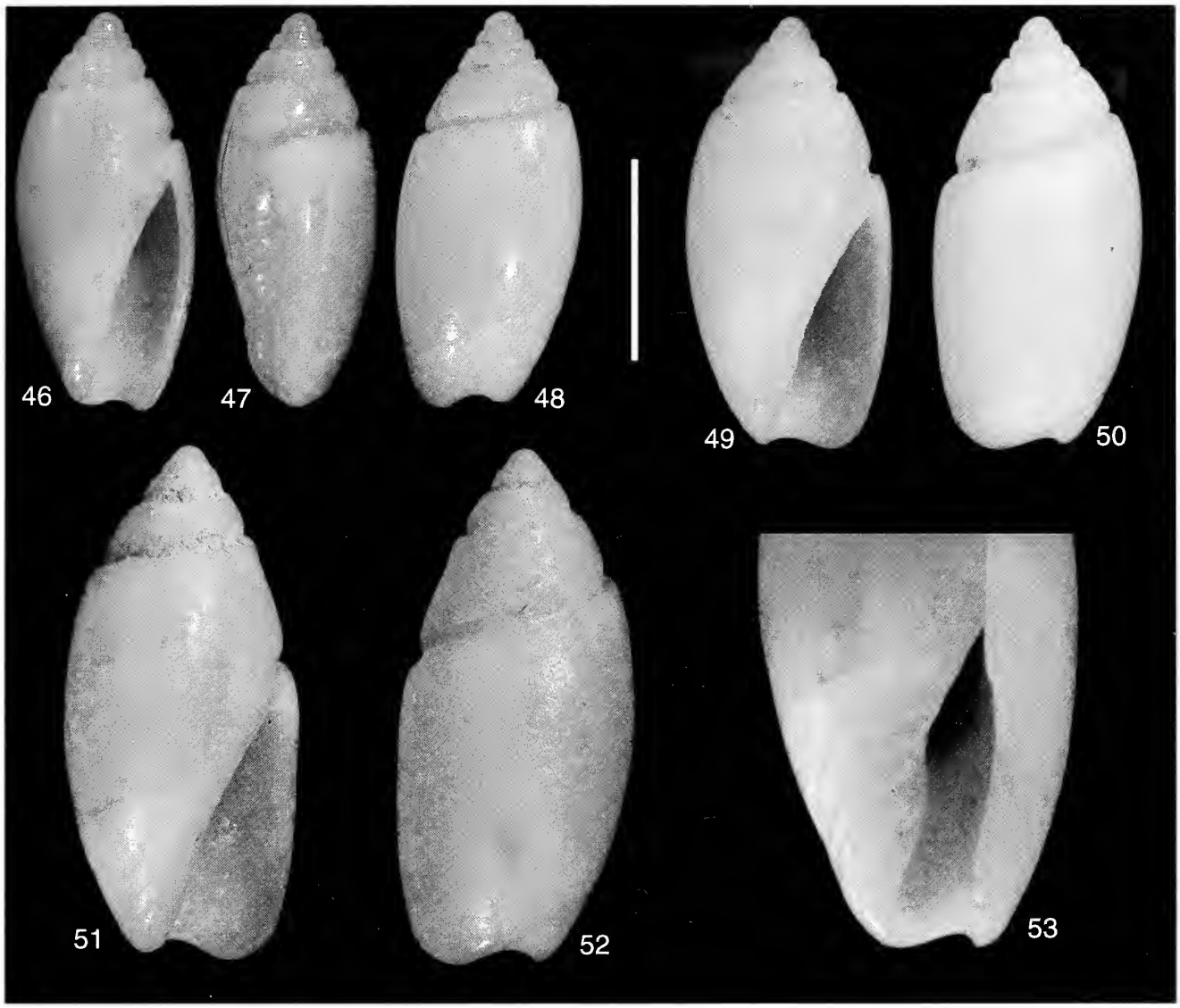
Distribution: Rio Grande do Sul, Brazil (Rios, 2009) to Punta Pardelas, Golfo Nuevo, Chubut, Argentina.

Remarks: *Olivella tehuelcha* and *O. puelcha* were eonfused sinee they were published initially by Duelos and later by d'Orbigny (see Remarks under *O. puelcha*). As it was stated by d'Orbigny (1840: 418, footnote), Duelos's illustration was transposed so the names were ehanged, but they have priority, which supports Duelos's original designation. Therefore, d'Orbigny's type material of *Olivina puelchana* refers to *Oliva tehuelcha* Duelos. Klappenbaeh (1991b) clarified the changes and different denominations of both species. As suggested previously by Klappenbach (1964), *O. defiorei* from Brazil is a eomparable speeies. He pointed out differenees in the eolor pattern and the absence of operculum in the Brazilian species. In the same paper he illustrated the raehidian tooth of the radula of *O. tehuelcha*. That illustration shows no intermediate, obsolete dentieles between the more developed, normal ones. In addition, in *Olivella defiorei* the dentieles end far from the tips of the raehidian. These eharacters are only visible at the SEM, therefore it is highly plausible that Klappenbach never saw them. Formiea-Corsi (1900: 80, fig. 19) illustrated in his catalogue of mollusks from Uruguay a somewhat wide specimen, which is closer to *Olivancillaria contortuplicata*. Nevertheless the description fits that of *Olivella tehuelcha*.

Olivella santacruzence Castellanos and Fernández, 1965
(Figures 46–53)

Olivella santacruzence Castellanos and Fernández, 1965: 102, fig. 10, 11.

Description: Shell small (up to 9 mm), subquadrangular, of 4–4.5 smooth whorls; Protoconch of about two whorls, without visible transition to teleoeoneh. Color white, rarely with some very weak yellow spots. Spire low, suture channeled, very wide. Paricta callus smooth, weakly developed. Columella with six oblique plaits (nine in the holotype, aecording to the authors). Faseiolar



Figures 46–53. *Olivella santacruzence* Castellanos and Fernández, 1965. **46–52.** MLP 3863 paratypes, Punta Medanosa, Santa Cruz province, Argentina. **53.** Detail of the columellar plaits of the shell in Figure 51. Scale bar, all shells = 3 mm.

band wide, distinctly colored, posterior groove well defined. Soft parts unknown.

Type Material: Six paratypes, MLP 3863 (incorrectly published by the authors as 27284). However, only five are referable to this species, as the sixth is a juvenile male of *O. puelcha*. None of these specimens match the published size of the holotype, which is apparently lost. All the specimens are beach-collected.

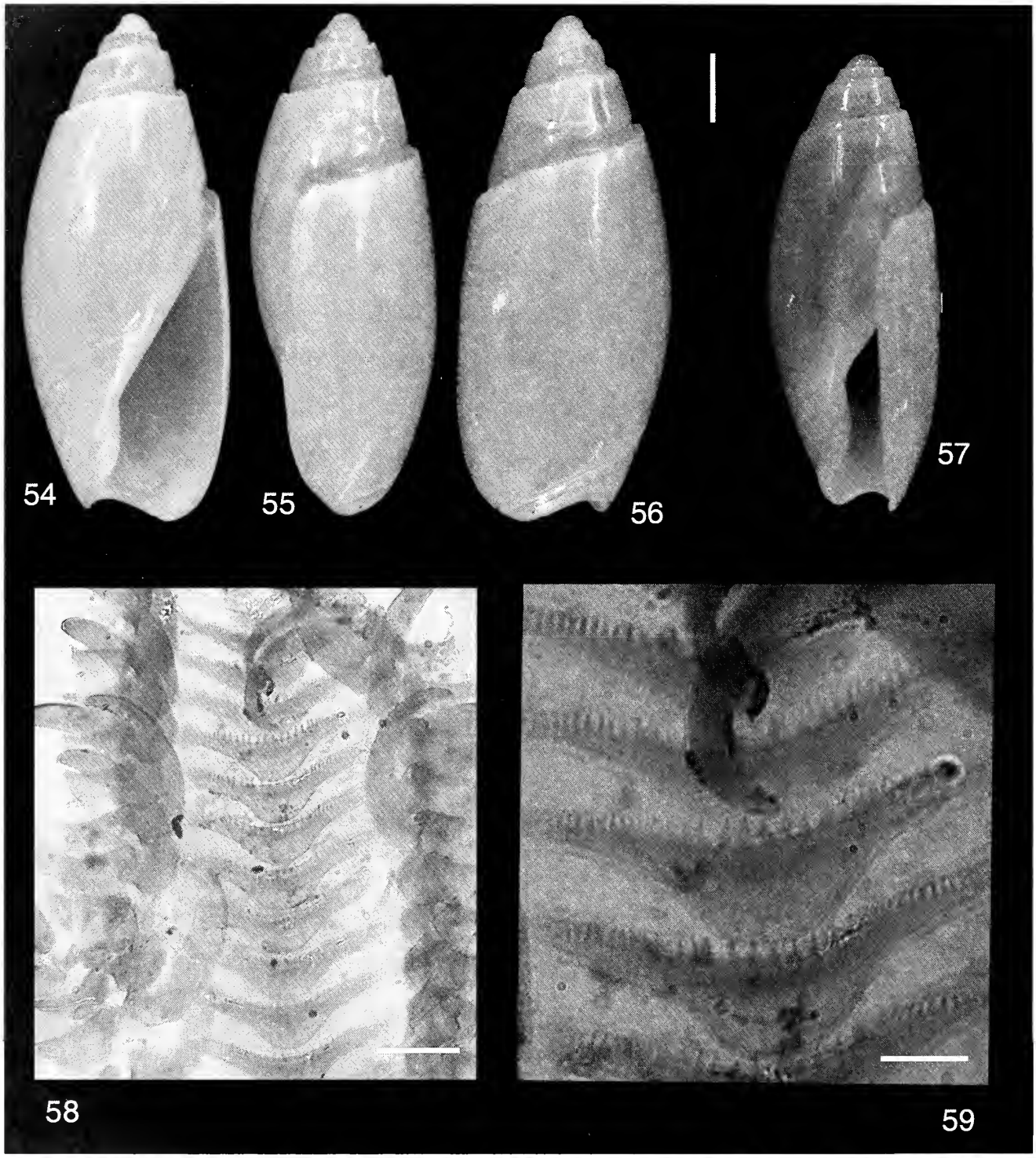
Type Locality: Punta Medanosa, Santa Cruz Province, Argentina (approximately 48°04′ S, 65°56′ W). This locality was recently visited, but specimens could not be found.

Distribution: Known only from the type locality.

Olivella orejasmirandai Klappenbach, 1986
(Figures 54–59)

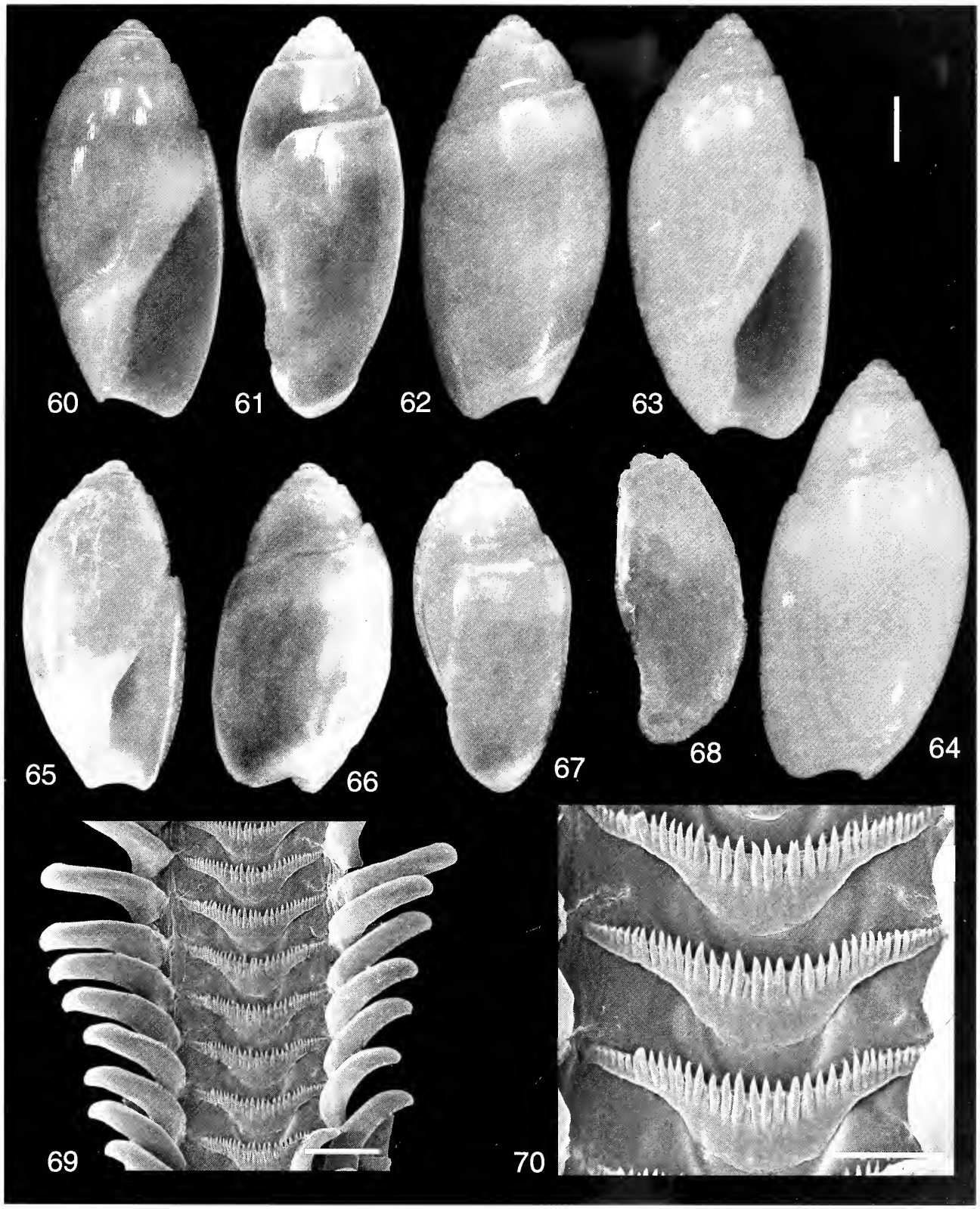
Olivella (Olivina) orejasmirandai Klappenbach, 1986: 2, figs. 1–5; Rios, 1994: 145, pl. 47, fig. 627.

Description: Shell small size for the genus, reaching 8 mm in length, elongated, solid, with five smooth, very flat whorls. Protoconch with 1.5 whorls, totally smooth; transition to teleoconch visible. Color whitish, some specimens translucent, with a subsutural weak white line. Spire elevated, conical, <0.5 total length; suture channeled, very deep and wide, with the margin reflected over canal; Columellar lip strong, well defined, with a sinuous abaxial margin. Parietal callus thick, growing adapically over suture, covering up to half of previous whorl. Parietal lip oblique,



Figures 54–59. *Oliviella oreasmirandai* Klappenbach, 1986. **54–56.** Holotype, MNHNM 14765, 33° 17′ S, 50° 34′ W, Off Albardão, Rio Grande do Sul State, Brazil. **57.** Paratype, MNHNM 14766, tilted 30° to show curved columella. **58.** Holotype, radula. **59.** Detail of rachidian teeth of same radula. Scale bars: Figure 57 = 1 mm; 58 = 50 µm; 59 = 20 µm.

straight, with sudden change of direction beyond columella. Outer lip sharp. Aperture triangular. Columella with only one plait. Fasciolar band white, posterior groove distinct, deep. Radula rachiglossate, with 22 rows of teeth. Rachidian teeth with strongly curved base; 38–40 denticles of same size along middle of rachidian, but getting thinner toward sides. Lateral teeth typically curved, sharp at end. A quadrangular piece is present under lateral teeth.



Figures 60–70. *Olivella* cf. *riosi* Klappenbach, 1991. **60–62.** MACN-In. 37602. 42°56′ S. 64°25′ W. 15 m depth, about 700 m off Estancia el Pedral Golfo Nuevo, Chubut, Argentina. **63–64.** Holotype of *O. riosi* Klappenbach, 1991. MNHNM 14773. 35°36′05″ S. 53°32′00″ W. **65–67.** Another specimen, same locality as for Figures 60–62. **68.** Operculum of the specimen in Figures 60–62. **69.** Radula, general view. **70.** Radula, rachidian teeth. Scale bars: All shells = 1 mm; Figure 69 = 50 µm; 70 = 30 µm.

Opereulum translueent, yellowish, elliptieal. Unfortunately, no soft parts are known other than radula and operculum.

Type Material: Holotype MNHNM 14765; paratypes, MNHNM 14766, 11017 and 14769.

Type Locality: Off Albardão, Rio Grande do Sul state, Brazil (33°17′ S, 50°34′ W) in 173 m. Paratypes: Off Rio de la Plata, Samborombón Bay.

Distribution: Southern Brazil, off Cabo Santa María, Uruguay, and off Río de la Plata, Argentina.

Remarks: This taxon is presently known only from the type material. The radulae were re-studied and illustrated from the original slides. It is somewhat different from what is depicted in the author's illustrations. Figures 58 and 59 show the dentieles of the raehidian of almost the same size in the middle of the teeth and larger than those from the tips.

Olivella cf. *riosi* Klappenbach, 1991
(Figures 60–70)

Olivella (Olivina) riosi Klappenbach, 1991a: 2, figs. 1–3, [7–10 in error in the original publication] 4, 5; 9–10.

Description: Shell very small for the genus, up to 6.5 mm, suboval, of 4–4.5 flat, smooth whorls; Protoeoneh short, number of whorls hard to determine, as there is no visible transition to the teleoconch. Color pale reddish or brownish with indistinet withish subsutural band. Spire very low, suture canalieulated, wide. Parietal eallus pronounced, smooth, well defined. Columella with one flat, wide plait. Faseiolar band wide, whitish; posterior groove obsolete.

Radula rachiglossate, with 18 rows of teeth. Rachidian teeth narrow, with small eurved base; 34–36 sharp denticles of irregular size along middle of each tooth, diminishing toward sides. Lateral teeth, long, typieally eurved, with blunt ends and flat profile. Under laterals a quadrangular pieee is present. Operculum extremely thin, translueid, subterminal nueleus. Growth lines covering entire surface.

Distribution: Known only from about 700 m off Estancia el Pedral, Golfo Nuevo, Chubut, Argentina (42°56′ S, 64°25′ W) in 15 m depth.

Remarks: These two specimens were compared to the type material of *O. riosi*. At present there is no clear way to separate the two speeies. However, as more material becomes available (male speeimens), this may prove to be a new speeies.

DISCUSSION

Four species of *Olivella* are presently known to oecur in Argentine waters. Of these, three are also reeorded from Uruguay and Brazil. It is clear that the genus is basically a temperate group so the diversity decreases in colder waters.

In his elassieal paper on the *Olivella* of North and Central America, Olsson (1956) reviewed a large number of species and established subgenera based primarily on shell and radular eharaeters. He included the two eommon species from Argentina, *O. puelcha* (as *O. tehuelchana*) and *O. tehuelcha* (as *O. puelchana*), as well as the northern *O. bullula*, in the subgenus *Olivina* d'Orbigny, for which *O. puelcha* Duclos (=*O. tehuelchana* d'Orbigny in the original) serves as type species. The main characters established by Olsson for the subgenus *Olivina* are: narrow sutures, and a low eolumella, with one or two folds. These characteres are aetually very variable, and some included speeies (e.g., *O. santacruzence*, with several columellar folds) do not conform to these eriteria. The presence of the operculum was eonsidered by Olsson to be a subgeneric charaeter. However, neither the morphology of the radulae nor of the penes were used to distinguish the subgenera.

My review of several of the South American speeies of *Olivella* shows that the morphology of the penis appears to be a distinguishing feature for taxonomic decisions. Most of the speeies deseribed here as well as some of the Brazilian species (e.g., *Olivella riosi*, *O. minuta*, *O. tehuelcha*, *O. puelcha*, *O. semistriata*, and *O. formicacorsii*) studied have an extremely eharaeteristie penis that allows for elear identifieation. In contrast, the morphology of the shell, which was used as a major tool to differentiate speeies or genera, is sometimes an unreliable souree of eharaeters. In addition, an interesting sexual dimorphism in shell morphology was reeently diseovered in *O. puelcha*, which raises doubts about the unequivoeal use of shell eharaeters to distinguish speeies or genera (see Pastorino, 2007). Unfortunately, samples that can be reliably sorted to by sex are not always available for study.

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Molecular data provide new insights on the phylogeny of the Conoidea (Neogastropoda)

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ABSTRACT

The superfamily Conoidea is one of the most speciose groups of marine molluscs, with almost 700 genera and 10,000 living species. Previous classifications were based on morphological and anatomical characters, but clades and phylogenetie relationships were not well assessed. Information provided by one mitochondrial (COI) and three nuclear (28S, 18S, and H3) genes were used to infer the phylogeny of this group. Data were obtained from more than 100 speeimens, belonging to 54 genera, collected during recent cruises in the western Pacific (Philippines, Vanuatu, Norfolk Ridge, and Chesterfield and Solomon Islands). Analyses were performed on eaeh gene independently as well as for a data matrix where all genes were concatenated, using several methods (ML, Parsimony, Bayesian). Some families and subfamilies among Conoidea eorrespond to well-supported clades uniformly recovered with all genes and all methods, but others appear to be polyphyletic. Several bathyal and abyssal genera are also shown to be polyphyletic. Our results also point out some new phylogenetie relationships at the family, subfamily, and genus levels.

Additional keywords: 18S rDNA, 28S rDNA, classifieation, COI gene, Conoidea, Conidae, H3 gene, molecular phylogeny, Toxoglossa, Turridae, western Pacific

INTRODUCTION

The superfamily Conoidea, or Toxoglossa, is one of the most prolific groups of marine molluscs, both in genera, with almost 700, and species, with perhaps 10,000 recent and fossil species (Bouchet, 1990). The genus *Conus* alone includes more than 500 species, making it the most speciose genus of marine animals (Kohn, 1990; Duda and Kohn, 2005). The monophyly of the group, characterized by a venom apparatus (Taylor et al., 1993), is not questioned, but the classification within Conoidea still remains problematic. Subdivisions within Toxoglossa and relationships between them are not well-defined, mostly because of the huge morphological and anatomical variation encountered.

During most of the 19th and 20th centuries, classifications (e.g., Fischer, 1887; Cossmann, 1896; Hedley, 1922; Thiele, 1929; Wenz, 1938–1944) were based on characters of the shell and of the radula, and Powell (1942, 1966) later gave emphasis on characters of the protoconch. All these authors traditionally recognized three families of Recent Conoidea: (1) Conidae, only containing the genus *Conus*, (2) Terebridae containing species with acuminate shells without a siphonal canal, and (3) Turridae, including the remainder, i.e., the vast majority of the group. Powell's (1942, 1966) subdivision of the Turridae in nine subfamilies was the basis for turrid classifications in the latter half of the 20th century. Subsequent authors diverged on the number of subfamilies they recognized, mostly splitting one subfamily into several (McLean, 1971; Kilburn, 1983, 1985, 1986, 1988, 1991, 1992, 1995). Taylor et al. (1993) extensively used anatomical characters, in addition to radulae, to

propose an entirely novel classification with six families (Conidae, Turridae, Terebridae, Drilliidae, Pseudomelatomidae, and Strictispiridae). The most important changes introduced in their classification were that Conidae was by then enlarged beyond Coninae (*Conus*) to include five subfamilies previously placed in Turridae, and that the newly restricted Turridae included five additional subfamilies. Bouchet and Rocroi's (2005) recent review of gastropod classification essentially retained Taylor's classification with updates based mainly on Rosenberg (1998) and Medinskaya and Sysoev (2003). We use "Turridae sensu lato" to designate all Conoidea except *Conus* and Terebridae (i.e., Turridae sensu Powell (1966) and most 20th century authors) and "Turridae sensu stricto" to designate the family as restricted by Taylor et al. (1993), while "Conidae" designates the expanded family after Taylor et al. (1993).

Although *Conus* itself has been subjected to intensive molecular studies (e.g., Duda and Kohn, 2005), the phylogeny of the broader Conoidea has not yet been addressed based on molecular characters. The present paper, which expands on our earlier work (Puillandre et al., 2008), presents the first molecular phylogeny based on one mitochondrial and three nuclear genes of the crown clade of the Caenogastropoda. It provides insights at several taxonomic levels (generic, subfamilial, and familial) and offers re-evaluations of the adequacy of previous classifications.

MATERIALS AND METHODS

Materials: A total of 108 specimens of Conoidea were used for molecular analyses, representing 54 valid generic names (Table 1). Eight specimens, noted *cf.*, could not be attributed with certainty to a genus. Specimens of Terebridae and *Conus* were identified to species level. Specimens were sampled during several cruises from 2004 to 2006 in the southwestern Pacific. Living specimens were anesthetized, a piece of tissue was cut from the head-foot, and fixed in 95% ethanol. Shells were kept intact for identification. A specimen of a species of *Nassaria* and a specimen of a species of *Cancellopollia*, both in the neogastropod family Buccinidae, closely related to Conoidea (Harasewych et al., 1997; Colgan et al., 2007), were used as outgroups. *Littorina littorea* (Linnaeus, 1758), belonging in the non-neogastropod family Littorinidae, was used as a third outgroup, with sequences taken from GenBank (GenBank accession numbers: AJ622946.1, Q279985.1, AJ488712.1 and DQ093507.1). Outgroups were chosen to form a non-monophyletic group, as recommended by Darlu and Tassy (1993). All vouchers are kept in MNHN.

Sequencing: DNA was extracted from a piece of foot, using 6100 Nucleic Acid Prepstation system (Applied Biosystem) or DNeasy® 96 Tissue kit (Qiagen) for smaller specimens. A fragment of 658 bp of Cytochrome OxidaseI (COI) mitochondrial gene was amplified using the universal primers LCO1490 and HCO2198 developed by Folmer et al. (1994). Three nuclear gene fragments were also analyzed: (1) 900 bp of the rDNA 28S gene, involving D1, D2 and D3 domains (Hassanna et al., 1984), using the primers C1 and D3 (Jovelin and Justine, 2001); (2) 328 bp of the H3 gene using the primers H3aF and H3aR (Okusu et al., 2003); (3) 1770 bp of the 18S gene using three pairs of primers: 1F and 5R, 3F and Bi, A2 and 9R (Giribet et al., 1996; Okusu et al., 2003). All PCR reactions were performed in 25 µl, containing 3 ng of DNA, 1X reaction buffer, 2.5 mM $MgCl_2$, 0.26 mM dNTP, 0.3 µM of each primer, 5% DMSO and 1.5 units of Q-Bio Taq (Qbiogene) for all genes. Amplifications consisted of an initial denaturation step at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 28S gene and first and third fragment of 18S gene, and 53°C for H3 gene and second fragment of 18S gene for 40 sec and extension at 72°C for 1 min. The final extension was at 72°C for 10 min. Thermocycles used for CO1 gene were described in Hebert et al. (2003). PCR products were purified and sequenced by the Genoscope (Genbank accession numbers: EU015417-EU015858).

Phylogenetic Analyses: COI and H3 genes were manually aligned whereas 28S and 18S genes were automatically aligned using ClustalW multiple alignments implemented in BioEdit version 7.0.5.3 (Hall, 1999). Nucleotide substitution models were selected for each gene separately and for each combined dataset using the program Modeltest (Posada and Crandall, 2001), in conjunction with PAUP 4.0b10 (Swofford, 2002). Analyses were conducted using three different approaches. A heuristic Maximum Parsimony (MP) search was executed with 100 Random Taxon-Addition (RA), Tree-Bisection and Reconnection (TBR) branch-swapping, all sites equally weighted and indels treated as fifth states, using PAUP 4.0b10 (Swofford, 2002). Maximum Likelihood (ML) heuristic search was conducted with 100 replicates with TBR branch-swapping using PhyML 2.4.4 (Guindon and Gascuel, 2003). Robustness of the nodes was assessed using nonparametric bootstrapping (Felsenstein, 1985) with 100 bootstraps replicates for MP analysis and 1000 for ML analysis, TBR branch-swapping and 100 RA replicates. Bayesian Analysis (BA) consisted of six Markov chains (8000000 generations each with a sampling frequency of one tree each hundred generations) run in two parallel analyses using Mr. Bayes (Huelsenbeck et al., 2001). For the treatment of combined data using BA, the data were separated into four different partitions corresponding to the four genes analyzed, each following the best fitting model of substitution estimated for each gene.

Phylogeny and Classification: Because of the instability of the taxonomy of the group, currently accepted synonymies cannot be taken for certain and must be re-evaluated. Our taxon sampling includes several genera for as many as possible of the subfamilies proposed in the literature (Table 2). From a nomenclatural perspective, only the occurrence of the type genus of a family-group name in a clade allows for an unequivocal application of this name to that clade. For example, the

Table 1. Specimens of Conoidea used in this study. Identification number (ID) and cruise of collection are given for each specimen. Specimens are identified to genus level, except *Conus* and Terebridae which are identified at species level. A cross indicates that the specimen was successfully sequenced for the gene. Allocation to clades A, B, C and 1 to 21, as defined by the molecular analysis, is given for each taxon.

ID	Cruise	Genus (or species) identification	COI	28S	18S	H3	Clades	
17700	BOA 1	*Bathytoma* Harris and Burrows, 1891	×	×	×	×	20	B
17701	BOA 1	*Leucosyrinx* Dall, 1889	×	×	×	×	9	A
17702	BOA 1	*Leucosyrinx* Dall, 1889	×	×	×	×	9	A
17754	Panglao 2004	*Turris* Röding, 1798	×	×	×	×	5	A
17755	Panglao 2004	*Crassispira* Swainson, 1840	×	×	×	×	2, C	A
17835	BOA 1	*Benthomangelia* Thiele, 1925	×	×	×	×	17	B
17836	BOA 1	*Rimosodaphnella* Cossmann, 1915	×	×	×	×	10	B
17837	EBISCO	*Inquisitor* Hedley, 1918	×	×	×	×	2, C	A
17838	EBISCO	*Gemmula* Weinkauff, 1875	×	×	×	×	5	A
17839	EBISCO	*Borsonia* Bellardi, 1839	×	×	×	×	16	B
17840	EBISCO	*Horaiclavus* Oyama, 1954	×	×	×	×	7	A
17841	EBISCO	*Gymnobela* Verrill, 1884	×	×	×	×	10	B
17842	EBISCO	*Cochlespira* Conrad, 1865	×	×	×	×	8	A
17843	EBISCO	*Funa* Kilburn, 1988	×	×	×	×	2, C	A
17844	EBISCO	*Gymnobela* Verrill, 1884	×	×	×	×	10	B
17845	EBISCO	*Teretiopsis* Kantor and Sysoev, 1989	×	×	×	×	10	B
17846	EBISCO	*Leucosyrinx* Dall, 1889	×	×	×	×	3, C	A
17847	EBISCO	*Splendrillia* Hedley, 1922	×	×	×	×	1, C	A
17848	EBISCO	*Pleurotomella* Verrill, 1873	×	×	×	×	10	B
17849	EBISCO	*cf. Gemmuloborsonia* Shuto, 1989	×	×	×	×		A
17850	EBISCO	*Turridrupa* Hedley, 1922	×	×	×	×	5	A
17851	EBISCO	*Inquisitor* Hedley, 1918	×	×	×	×	2, C	A
17852	EBISCO	*Gemmula* Weinkauff, 1875	×	×	×	×	5	A
17853	EBISCO	*Heteroturris* Powell, 1967	×	×	×	×	18	B
17855	Norfolk 2	*Benthofascis* Iredale, 1936		×	×	×		B
17857	EBISCO	*Bathytoma* Harris and Burrows, 1891	×	×	×	×	20	B
17858	Panglao 2004	*Clavus* Monfort, 1810	×	×	×	×	1, C	A
17859	Panglao 2004	*Turridrupa* Hedley, 1922	×	×	×	×	5	A
17860	Panglao 2004	*Lophiotoma* Casey, 1904	×	×	×	×	5	B
17861	Panglao 2004	*Kermia* Oliver, 1915	×	×	×	×	10	B
17862	Panglao 2004	*Gemmula* Weinkauff, 1875	×	×	×	×	5	A
17863	Panglao 2004	*Macteola* Hedley, 1918	×	×	×	×	11	B
17864	Panglao 2004	*cf. Guraleus* Hedley, 1918	×	×	×	×	11	B
17865	Panglao 2004	*Bathytoma* Harris and Burrows, 1891	×	×	×	×	20	B
17866	Panglao 2004	*Mangelia* Risso, 1826	×	×	×	×	11	B
17867	Panglao 2004	*Borsonia* Bellardi, 1839	×	×	×	×	16	B
17868	Panglao 2004	*Anacithara* Hedley, 1922	×	×	×	×	7	A
17869	Panglao 2004	*Etrema* Hedley, 1918	×	×	×	×	12	B
17870	Panglao 2004	*Otitoma* Jousseaume, 1898	×	×	×	×	2, C	A
17871	Panglao 2004	*Kermia* Oliver, 1915	×	×	×	×	10	B
17872	Panglao 2004	*Macteola* Hedley, 1918	×	×	×	×	11	B
17873	Panglao 2004	*Guraleus* Hedley, 1918	×	×	×	×	11	B
17874	Panglao 2004	*Guraleus* Hedley, 1918	×	×	×	×	11	B
17875	Panglao 2004	*Tomopleura* Casey, 1924	×	×	×	×	14	B
17876	Panglao 2004	*Lienardia* Jousseaume, 1928	×	×	×	×	12	B
17877	Panglao 2004	*Mitromorpha* Carpenter, 1865	×	×	×	×	13	B
17878	Panglao 2004	*Kermia* Oliver, 1915	×	×	×	×	10	B
17879	Panglao 2004	*Inquisitor* Hedley, 1918	×	×	×	×	2, C	A
17880	Panglao 2004	*Kermia* Oliver, 1915	×	×	×	×	10	B
17881	Panglao 2004	*Daphnella* Hinds, 1844	×	×	×	×	10	B
17882	Panglao 2004	*Raphitoma* Bellardi, 1848	×	×	×	×	10	B
17883	Panglao 2004	*Veprecula* Melvill, 1917	×	×	×	×	10	B
17884	Panglao 2004	*Leiocithara* Hedley, 1922	×	×	×	×	11	B
17885	Panglao 2004	*Ceritoturris* Dall, 1924	×	×	×	×	7	A
17886	Panglao 2004	*Splendrillia* Hedley, 1922	×	×	×	×	1, C	A
17887	Panglao 2004	*Microdrillia* Casey, 1903	×	×	×	×	18	B
17888	Panglao 2004	*Ceritoturris* Dall, 1924	×	×	×	×	7	A
17889	Panglao 2004	*Conopleura* Hinds, 1844	×	×	×	×	1, C	A

(Continued)

Table 1. (Continued)

ID	Cruise	Genus (or species) identification	COI	28S	18S	H3	Clades	
17890	Panglao 2004	*Raphitoma* Bellardi, 1848	×	×	×	×	10	B
17891	Panglao 2004	*cf. Tritonoturris* Dall, 1924	×	×	×	×	10	B
17892	Panglao 2004	*cf. Glyphostomoides* Shuto, 1983	×	×	×	×	10	B
17893	Panglao 2004	*cf. Mitromorpha* Carpenter, 1865	×	×	×	×	13	B
17894	Panglao 2004	*Lienardia* Jousseaume, 1928	×	×	×	×	12	B
17895	Panglao 2004	*Inquisitor* Hedley, 1918	×	×	×	×	2, C	A
17896	Panglao 2004	*Eucithara* Fischer, 1883	×	×	×	×	11	B
17897	Panglao 2004	*Lienardia* Jousseaume, 1928	×	×	×	×	12	B
17898	Panglao 2004	*Mitromorpha* Carpenter, 1865	×	×	×	×	13	B
17899	Panglao 2004	*Eucithara* Fischer, 1883	×	×	×	×	11	B
17900	Panglao 2004	*Eucithara* Fischer, 1883	×	×	×	×	11	B
17901	Panglao 2004	*Anarithma* Iredale, 1916	×	×	×	×	13	B
17902	Panglao 2004	*Clavus* Monfort, 1810	×	×	×	×	1, C	A
17903	Panglao 2004	*Eucyclotoma* Boettger, 1895	×	×	×	×	10	B
17904	Panglao 2004	*cf. Nannodiella* Dall, 1919	×	×	×	×	12	B
17905	Panglao 2005	*Otitoma* Jousseaume, 1898	×	×	×	×	2, C	A
17906	Panglao 2005	*Ptychobela* Thiele, 1925	×	×	×	×	2, C	A
17907	Panglao 2005	*Gemmula* Weinkauff, 1875	×	×	×	×	5	A
17908	Panglao 2005	*Iwaoa* Kuroda, 1953	×	×	×	×	7	A
17909	Panglao 2005	*Cinguloterebra cf. fujitai* Kuroda and Habe, 1952	×	×	×	×	6	A
17910	Panglao 2005	*Tomopleura* Casey, 1924	×	×	×	×	14	B
17911	Panglao 2005	*cf. Heteroturris* Powell, 1967	×	×	×	×	18	B
17912	Panglao 2005	*Conus praecellens* Adams, 1854	×	×	×	×	19	B
17913	Panglao 2005	*Conus sulcatus* Hwass in Bruguière, 1792	×	×	×	×	19	B
17914	Panglao 2005	*Conus sulcatus* Hwass in Bruguière, 1792	×	×	×	×	21	B
17915	Panglao 2005	*Toxicochlespira* Sysoev and Kantor, 1990	×	×	×	×	17	B
17916	Panglao 2005	*Comitas* Finlay, 1926	×	×	×	×	4, C	A
17917	Panglao 2005	*Terebra polygyrata* Deshayes, 1859	×	×	×	×	6	A
17918	Panglao 2005	*Comitas* Finlay, 1926	×	×	×	×	4, C	A
17919	Panglao 2005	*Cochlespira* Conrad, 1865	×	×	×		8	A
17920	Panglao 2005	*Cochlespira* Conrad, 1865	×	×	×		8	A
17921	Panglao 2005	*Conus orbignyi* Kilburn, 1975	×	×	×	×	21	B
17922	Panglao 2005	*Conus wakayamaensis* Kuroda, 1956	×	×	×	×	21	B
17923	Panglao 2005	*Cinguloterebra cf. fenestrata* Hinds, 1844	×	×	×	×	6	A
17924	Salomon 2	*Thatcheria* Angas, 1877	×	×	×	×	10	B
17925	Salomon 2	*Toxicochlespira* Sysoev and Kantor, 1990	×	×	×	×	17	B
17926	Salomon 2	*Borsonia* Bellardi, 1839	×	×	×	×	15	B
17927	Salomon 2	*Daphnella* Hinds, 1844	×	×	×	×	10	B
17928	Salomon 2	*Comitas* Finlay, 1926	×	×	×	×	3, C	A
17929	Salomon 2	*Bathytoma* Harris and Burrows, 1891	×	×	×	×	20	B
17930	Salomon 2	*Benthomangelia* Thiele, 1925	×	×	×	×	17	B
17931	Salomon 2	*cf. Typhlomangelia* Sars, 1878	×	×	×	×	18	B
17932	Salomon 2	*Borsonia* Bellardi, 1839	×	×	×	×	15	B
17933	Salomon 2	*Comitas* Finlay, 1926	×	×	×	×	3, C	A
17934	Salomon 2	*Borsonia* Bellardi, 1839	×	×	×	×	16	B
17935	Salomon 2	*Inquisitor* Hedley, 1918	×	×	×	×	2, C	A
17936	Santo 2006	*Conus generalis* Linne, 1758	×	×	×	×	19	B
17937	Santo 2006	*Conus gauguini* Richard and Salvat, 1973	×	×	×	×	19	B
17938	Santo 2006	*Terebra textilis* Hinds, 1844	×	×	×	×	6	A
17939	Santo 2006	*Conus consors* Sowerby, 1833	×	×	×	×	19	B
17854	Norfolk 2	*Nassaria*, Buccinidae	×	×	×	×		
17856	Norfolk 2	*Cancellopollia*, Buccinidae	×	×	×	×		
GenBank		*Littorina*, Littorinidae	×	×	×	×		

clade containing the genus *Raphitoma* can unambiguously carry the name Raphitominae. However, many type genera are not represented in our taxon sampling and some of our molecular clades do not include a type genus. In such cases, we have relied on the traditional allocation of non-type genera to a subfamily to link clade and name. For example, a clade containing three genera classically classified in the family Drilliidae (Taylor et al., 1993; Tippet and Tucker, 1995) can carry the name Drilliidae, even though *Drillia* itself is not part of our taxon

sampling. However, this approaeh does not lead to an unequivoeal applieation of names when genera (or subfamilies) as traditionally eonstrued prove to be nonmonophyletie; in that ease, only the type speeies (or the type genus) is the legitimate bearer of the name.

RESULTS

Almost all speeimens were sequenced for the four genes (see details in Table 1). Saturation analyses for the two protein-coding genes revealed that the COI gene was highly saturated at the third codon position; aecordingly, we used only the first and seeond positions in the phylogenetie analyses. Independent analyses of each of the four genes provided very poorly resolved trees, with few well-supported clades (results not shown). Since no ineongrueney was revealed among the single gene analyses, we eonstrueted a combined dataset eomprising the data of the four gene fragments resulting in a sequence length of 3428 bp, ineluding 108 ingroups.

The Conoidea were found to be monophyletic, although not strongly supported (MP and ML bootstraps respectively: 65 and 79, Posterior Probabilities PP: 1). Within the Conoidea, two clades eould be distinguished: clade A (MP bootstraps: 58, ML bootstraps: 68, PP: 0.73) and elade B (MP bootstraps: 28, ML bootstraps: 52, PP: 1). Within the elade A, the clade C is found strongly supported with ML bootstraps (91) and PP (1). Analysis of the eombined datasets allowed the definition of 21 higher level clades, each of them strongly supported: MP and ML bootstraps > 80 and PP > 0.99 (Mason-Gamer and Kellogg, 1996; Zander, 2004). They ineluded from one to 12 genera eaeh (Figure 1, Table 2). Clades were numbered aecording to their position in the tree. Clades 1 to 9 are ineluded in clade A, and among them elades 1 to 4 are ineluded in elade C. Clades 10 to 21 are included in clade B.

All representatives of a genus clustered together in one of the 22 elades, exeept for representatives of *Borsonia*, *Comitas*, *Conus*, and *Lcucosyrinx*. The representatives of *Borsonia* and *Conus* split respeetively in clades 15–16 and 19–21, eaeh including only specimens from a single genus. The relationships between the two elades were not resolved and thus the monophyly of each of these genera cannot be rejected. Conversely, the monophyly of genera *Lcucosyrinx* and *Comitas* (clades 3, 4 and 9) can be rejected, since representatives of the two genera clustered in the elade 4.

DISCUSSION

Classification of the Conoidea: Although not strongly supported, our analysis suggests that the superfamily Conoidea is monophyletic. However, the Conoidea and two outgroups used here (*Cancellopollia* and *Nassaria*) both belong in the Neogastropoda, a group for which the phylogeny is not well resolved (Harasewyeh et al., 1997; Colgan et al., 2007), and the monophyly observed here eould thus be an artifact due to under-sampling within Neogastropoda. Within Conoidea, the large amount of diversity included in our dataset allows us to diseuss the eurrent elassifieation at genus, subfamily, and family levels.

Accuracy of Taxonomic Delimitations at the Genus Level: The genus is the lowest level for whieh we ean diseuss taxonomie delimitations sinee most of our speeimens are not identified at species level. Among the 54 genera identified in our dataset, monophyly ean be rejeeted for only two of them (*Lcucosyrinx* and *Comitas*), whieh indicates that in most eases shell morphology is an appropriate predictor of generie alloeations. Two further genera (*Borsonia* and *Conus*) are found to be diphyletie, but the position of the two defined elades is unresolved and thus monophyly eannot be exeluded.

Position of the Genera within the Subfamilies: Our analysis eonfirms many previous assignments of genera to subfamilies as in Taylor et al. (1993) and subsequent refinements of their elassifieation (Table 2). However several results do not eonfirm established classifieations. For example, the genus *Otitoma*, tentatively retained by in the Mangeliinae by Kilburn (2004), who acted based on shell charaeters, is here alloeated to the Crassispirinae.

Robustness of Subfamilial Delimitations: We found diserepaneies between our phylogeny and previous elassifications at the subfamily level. Thus, crassispirine genera are present in two clades (2 and 7), one of them (clade 2) eontaining the type genus. The polyphyly of this subfamily is supported by the existence of clade C, which includes clade 2, but excludes clade 7. Given that the relationships between elade 7 and others clades within elade A are not resolved, it is ineonclusive whether elade 7 must be ranked as its own subfamily or whether it must be grouped together with another existing subfamily. Similarly, the subfamily Cochlespirinae as eurrently eonstrued appears polyphyletie. In three eases (Mangeliinae, Coninae, Clathurellinae), polyphyly is possible but not demonstrated beeause of a general lack of support for deeper nodes in clade B.

Robustness of Familial Delimitations: Finally, our results also permit a diseussion of family classification within Conoidea. Taylor et al.'s (1993) anatomieal study suggested a closer relationship of Clathurellinae, Conorbinae, Mangeliinae, Oenopotinae, and Raphitominae to *Conus* than to other members of the family Turridae sensu lato and their extension of Conidae ineluded these turrid subfamilies. In our study, elade B, although weakly supported, eorresponds to Taylor et al.'s (1993) family Conidae.

Our study also revealed another weakly supported deep clade (clade A) that ineludes genera classified by Taylor et al. (1993) in three different families: Drilliidae, Terebridae and Turridae sensu stricto (consisting of Clavatulinae, Coehlespirinae, Crassispirinae, Turrinae and Zonulispirinae). Genera of the family Drilliidae

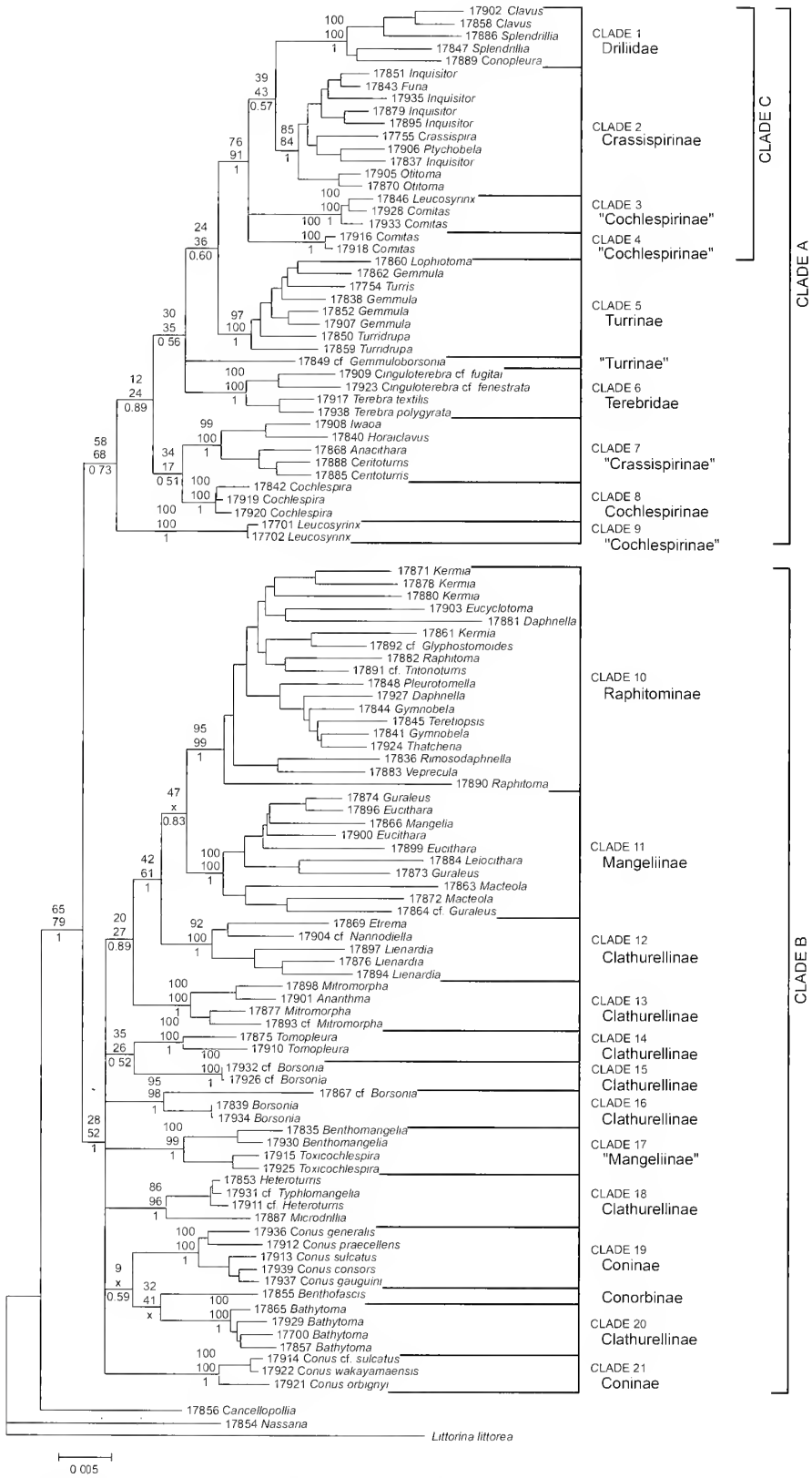
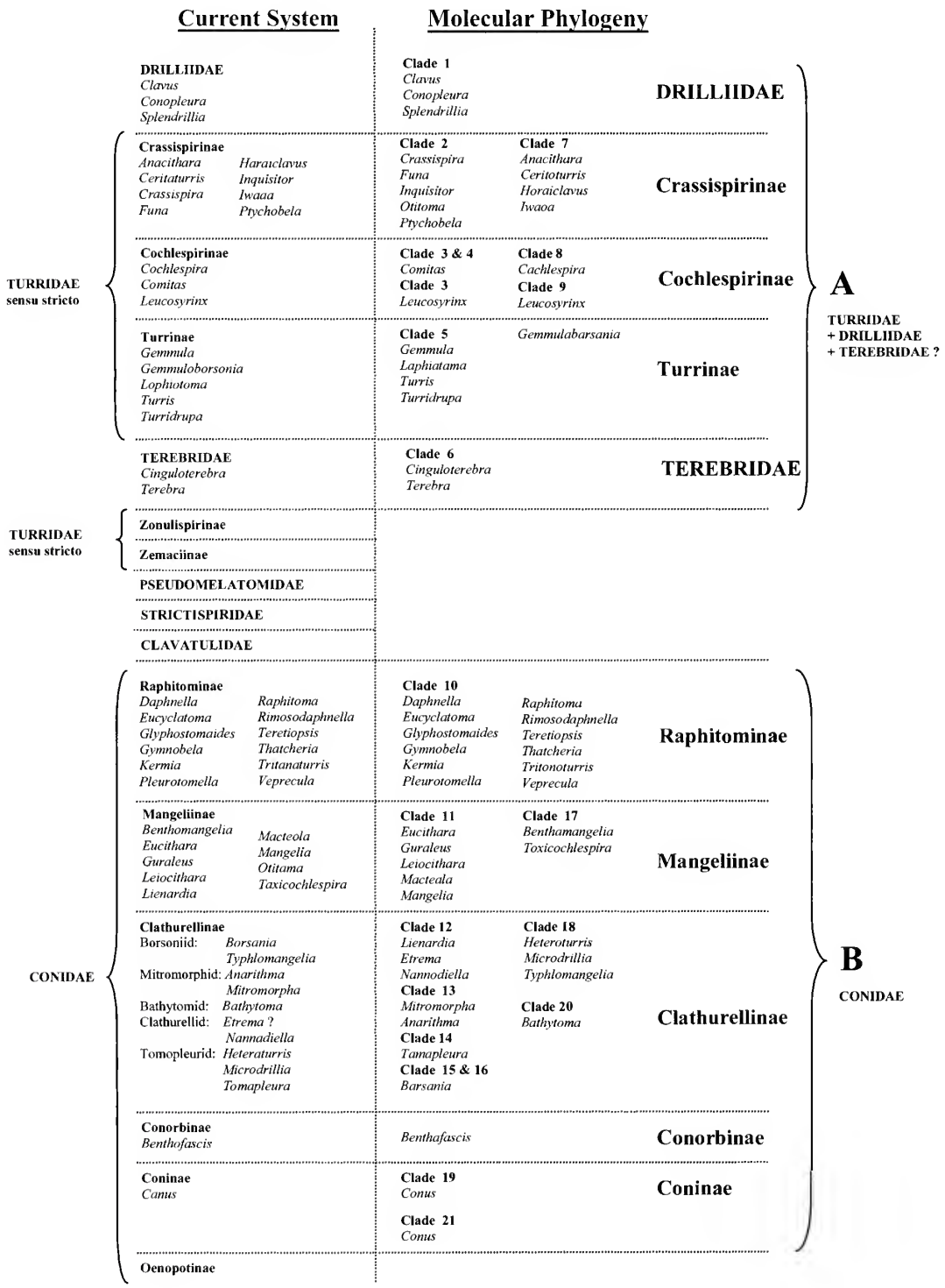


Figure 1. Consensus tree of MP, ML and BA. Nodes presented here were found with at least two of the three methods used. Top downwards, MP bootstraps, ML bootstraps and Posterior Probabilities are specified for each node. Support for intranodes of clades 1 to 21 are not presented.

Table 2. Current Conoidea classification and comparison with our results. Current Conoidea classification including genera used in the present study (based mainly on Taylor et al., 1993) and clades defined by the molecular phylogeny. Subfamilies are in bold, families in bold and capital.



(clade 1) are included in clade C. This well-supported clade also contains taxa of the family Turridae sensu stricto (Crassispirinae and *Comitas*), and excludes the other taxa of the family Turridae "sensu stricto" Consequently, Turridae sensu stricto are not monophyletic. Furthermore, according to Kantor (2006), the radula of Drilliidae is not fundamentally different from that of Turridae sensu stricto. Both our molecular data and this morphological evidence suggest that Drilliidae should be subsumed as a subfamily within the Turridae sensu stricto.

Within clade A, the monophyly of the family Terebridae is supported but its relationships with other clades of Turridae sensu stricto is not resolved. However, this result suggests that Terebridae are closely related to Turridae sensu stricto, as already proposed by Cossmann (1896), and Powell (1942; 1966).

Toward a Stabilized System for Conoidea: The taxonomic sampling used here allows for an estimate of molecular variability within clades at each level: several genera are included in each subfamily, several subfamilies are included in each family, and most of the families defined by Taylor et al. (1993) are present. However, even with a dataset of 54 genera, covering most of the previously recognized families and subfamilies of Conoidea, the present study only brings preliminary results. At genus level, these 54 genera represent only 16% of the 340 Recent genera described. It is clear that the shell-based current taxonomic definition of many genera will not stand after molecular testing. At subfamily and family levels, although a large part of the conoidean diversity is represented in this study, some families and subfamilies are not part of our taxon sampling. The highly divergent clades found here in several subfamilies as previously defined demonstrate the need for further research, which could better circumscribe subfamilies already known and probably formally name new subfamilies and/or tribes. Finally new relationships are suggested at the family level. As a remake of the *Conus* story, it now appears that the long recognized family Terebridae does not stand alone apart from the rest of the Conoidea, but could be the sister-group or even part of the Turridae sensu stricto.

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Proboscis and foregut morphology of *Ficus subintermedia* (d'Orbigny, 1852) (Caenogastropoda: Ficidae)

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ABSTRACT

Fig shells (Ficidae) have been identified as a putative sister group to Neogastropoda, although they have historically been associated with Tonnoidea. This study examines the morphology of the proboscis and foregut of *Ficus subintermedia* (d'Orbigny, 1852) and compares its major features to those of Neogastropoda and Tonnoidea. The elongate ficid proboscis is operated by an unusual arrangement of proboscis retractor muscles that connect to the esophagus and form a sheath around the proboscis. It appears that the proboscis can not be fully everted and is a functional analogue of an intraembolic proboscis, although this requires confirmation by observation of living animals. The salivary glands are shown to be superficially bilobed but histologically uniform, and the esophageal gland is minimally septate and confluent with the esophagus. Despite a morphologically complex alimentary system, there are few synapomorphies uniting Ficoidea with either Tonnoidea or Neogastropoda.

Additional keywords: Ficoidea, histology, anatomy, alimentary system, intraembolic proboscis

INTRODUCTION

The Ficidae, or fig shells, are a small family of marine caenogastropods that occupy benthic habitat across a global, mainly tropical, distribution. Despite their relatively large body size, moderate abundance and putative relationship to other well-studied caenogastropods, very little is known of the anatomy, systematics, life history, behaviour, or ecology of ficid species. The family Ficidae Conrad, 1867, was established exclusively for the genus *Ficus* Röding, 1798, within Tonnoidea. The subsequent systematic history of the group includes recognition of the superfamily Ficoidea Meek, 1864, the affiliation of Thalassocynidae Riedel, 1994 (containing *Thalassocyon* Barnard, 1960 [Beu, 1969]) with Ficoidea and the description of several ficid fossil genera (see Riedel, 1994).

Only a handful of studies have examined ficid morphology. Excluding descriptions of the shell, superficial examinations of the alimentary system (Amaudrut, 1898; Riedel, 1994), external morphology (Arakawa and Hayashi, 1972), mantle (Liu and Wang, 1996), nervous system (Bouvier, 1887) and radula (Warén and Bouchet, 1990; Riedel, 1994) are scattered throughout the literature. These data suggested to some reviewers (Warén and Bouchet, 1990; Riedel, 1994) that Ficoidea are morphologically distinct from Tonnoidea, but are insufficient to establish their relationship with other groups of caenogastropods.

The position of Ficidae within Caenogastropoda was examined by a combined morphological and molecular analysis (Riedel, 2000), which suggested Ficidae may be a sister taxon to Neogastropoda, united by features such as egg mass morphology, radular configuration, concentration of the circumesophageal nervous system, and operation of the proboscis. A more recent phylogeny of Caenogastropoda, using morphological data, placed Ficidae outside a large clade including the predatory groups Neogastropoda, Tonnoidea, and Cypraeoidea (Ponder et al., 2008). However, both these analyses were based on minimal and uncorroborated descriptions of ficid anatomy.

The internal relationships and evolution of Neogastropoda are a topic of considerable interest (Ponder, 1974; Taylor and Morris, 1988; Kantor, 1996; Harasewych et al, 1997; Kantor, 2002), but there is uncertainty surrounding the identity of extant sister taxa, the resolution of which would greatly assist in resolving internal neogastropod relationships by polarizing key morphological characters. Previous morphological studies have indicated that Ficoidea (Riedel, 2000), Tonnoidea (Graham, 1941; Ponder et al., 2008), a lower caenogastropod (Ponder, 1974; Golikov and Starobogatov, 1988), an epitoniid (Strong, 2003) or an underived carnivorous sorbeoconch (Kantor, 2002) is most closely related to Neogastropoda. There may be multiple sister taxa, as some authors consider Neogastropoda paraphyletic (see review by Taylor and Morris, 1988).

Further information on the morphology of Ficidae will be valuable in determining if they have synapomorphies which unite this group with either Neogastropoda or Tonnoidea. This study describes aspects of the anatomy and histology of *Ficus subintermedia* (d'Orbigny, 1852). The study focuses on the proboscis and foregut, as these structures are particularly informative in

defining groups of higher caenogastropods, including Neogastropoda.

MATERIALS AND METHODS

Specimens of *Ficus subintermedia* were obtained from the Australian Museum collections (C.353111). The specimens were collected by I. Loch at Cairns Reef, Queensland, Australia (15°42′ S, 145°30′ E) on 27 July 1973 and preserved in 5% formalin. Two male specimens were dissected under a stereo microscope and illustrated using a camera lucida. A third male specimen was post-fixed for 24 h in Bouin's fluid, dehydrated and saturated with Paraplast ™ paraffin using a Tissue-Tek ® VIP tissue processor. The embedded specimen was serially sectioned at 7 µm using an American Optical microtome. Mounted sections were stained using Cason's trichrome (acid fuchsin, aniline blue, and orange G) and Mayer's haematoxylin. Photographs of the sections were obtained using an Olympus DP70 digital camera mounted on an Olympus BX50 microscope.

The proboscis, salivary gland, jaws, and radula were removed from dissected specimens for scanning electron microscopy (SEM). The soft-tissue samples were dehydrated to 100% EtOH and critical point dried using a Bal-Tec CPD030. The radula was cleaned overnight using warmed NaOH to remove buccal tissue. The samples were sputter-coated with gold and examined using a Zeiss Evo LS15 SEM with a Robinson backscatter detector.

RESULTS

General Foregut Morphology: Foregut dominated by extremely long proboscis, ~2–3 times anterior esophagus length (from esophageal gland to buccal mass) (Figures 1–3, **pb**). Concentrated circumesophageal nerve ring anchors esophagus to pedal musculature anterior to esophageal gland. Introverted proboscis forms loop leading posteriorly through haemocoel to small buccal mass (Figure 2). Proboscis folded to occupy most of haemocoel, fused to lateral walls of head and foot at base of neck (Figure 3). Walls of slender neck form rhynchodeum with rhynchostome at tip (Figure 1, **rh**). Pair of large, tapering cephalic tentacles present on rhynchodeal wall; with subdermal, pigmented eyes at base (Figure 1, **ey**, **te**).

Proboscis: Fully introverted proboscis forms 'acrembolic' arrangement (Fretter and Graham, 1962); buccal mass and esophagus situated posterior to distal tip of proboscis (Figure 2). Walls of proboscis relatively thin (Figure 4, **pw**). Pair of nerves run laterally along internal surface of proboscis wall (=outer surface when introverted), each embedded in narrow sheet of circular muscle fibers that joins proboscis wall in two places (Figures 4, 5, **pn**, **cm**). When introverted, lateral proboscis wall pinched off by sheet of circular muscle to form two longitudinal flaps (here termed 'proboscis folds') which project into lumen of introverted proboscis (Figures 2, 4, 7, **pf**). Proboscis folds flattened when proboscis is everted; sheet of circular muscle stretched to accommodate greater circumference (Figure 8). Exterior surface of proboscis wall (=interior surface when introverted) covered with papillose epithelium, tallest on ventral surface, reduced in height on apex of each proboscis fold (Figures 7–9, **pa**). Each papilla approximately 50 µm in diameter, dotted with pores on tip (Figure 9). Histology of papillae composed of mucus cells opening to each pore, below extracellular cuticle layer.

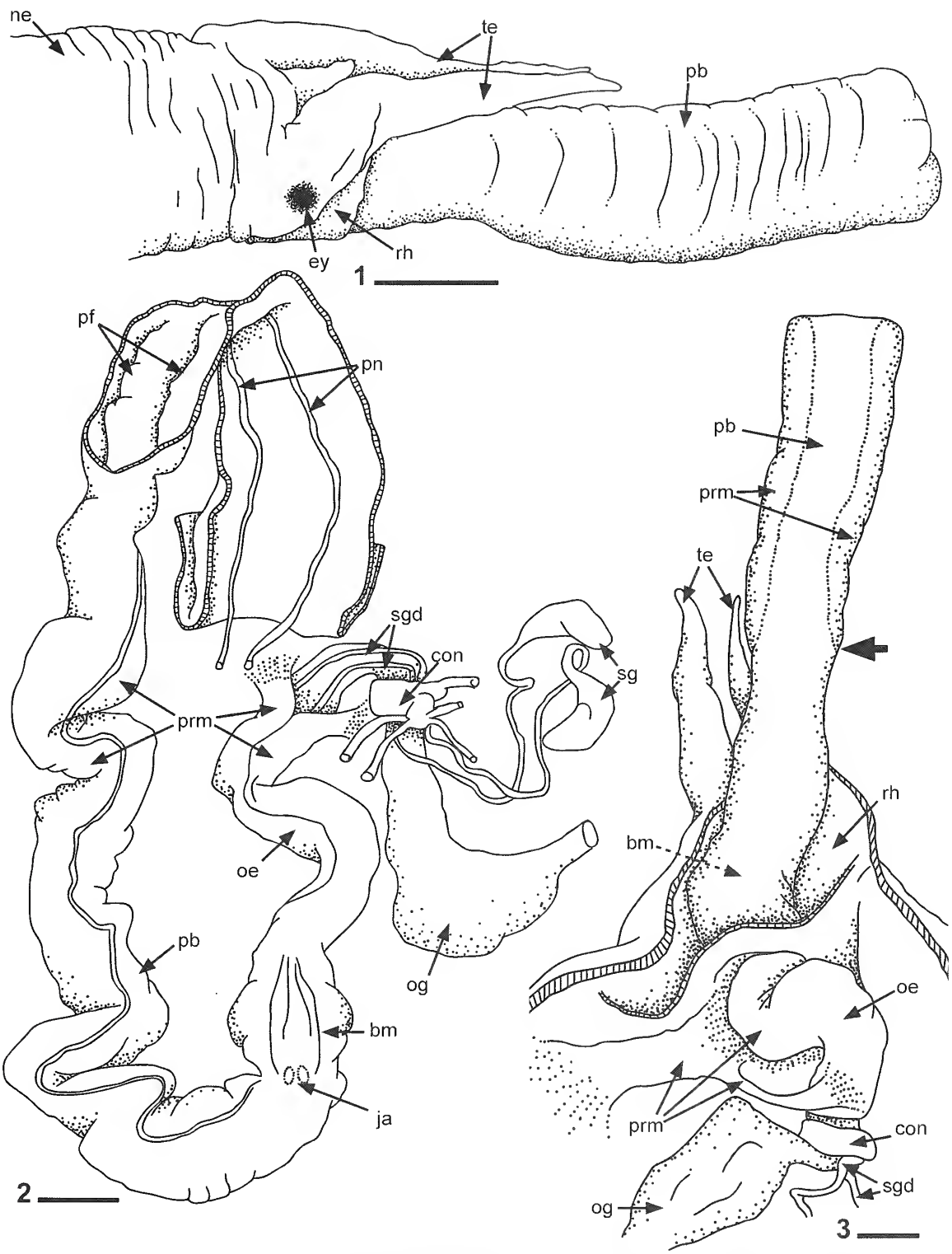
Pair of large retractor muscles attach to proboscis, anchor to lateral body walls (Figures 2, 3, **prm**). Proboscis retractor muscles short, fused to interior part of proximal proboscis wall near connection to rhynchodeum. Separate branch from each retractor muscle also connects to esophageal wall where it loops towards anterior of haemocoel to pass anterior to circumesophageal nerve ring (Figures 3, 6). Junction between proboscis retractors, esophagus situated approximately midway between esophageal gland and buccal mass (immediately anterior to circumesophageal nerve ring). Branches of retractor muscles extend anteriorly as two flattened sheets of longitudinal muscle sheathing dorsal, ventral surfaces of esophagus, buccal mass, salivary gland ducts, nerves situated near esophagus (Figures 2–6, **prm**). Near proximal proboscis base, retractor muscles taper off, fuse to inner wall of proboscis (Figure 3).

Buccal Mass and Radula: Buccal mass short, slightly wider than adjacent esophagus, proboscis (Figure 2, **bm**). Odontophoral retractor muscles derived from buccal mass inserted into proboscis retractor muscle covering esophagus. Pair of elliptical jaws present on dorsal surface of anterior buccal mass (Figure 2, **ja**). Jaws prismatic, composed of parallel rods (Figure 10).

Radula taenioglossan, similar to those figured by Riedel (1994) and Warén and Bouchet (1990) (Figure 11). Central tooth triangular, with large median cusp flanked on each side by six or seven secondary cusps (Figure 12). Each lateral tooth with major cusp directed centrally, single inner cusp, approximately 6 outer cusps of decreasing height. Both marginal teeth elongate, hook-shaped, inner marginal tooth differentiated with row of small cusps on outer edge (Figure 12).

Anterior Esophagus: Epithelium lining anterior esophagus folded, without any prominent or persistent longitudinal folds (Figures 13, 14, **oe**). No distinguishable dorsal, ventrolateral folds in posterior buccal mass or elsewhere in esophagus. Muscular esophageal wall composed of internal layer of ciliated columnar epithelium with occasional mucus cells, layer of longitudinal muscle, thick exterior layer of circular muscle (Figures 6, 13, 14).

Esophageal Gland: Posterior to nerve ring, esophagus expands to form esophageal gland (Figure 2, **og**). Histological sections through gland show epithelium not well preserved, but condition sufficient to determine main morphological aspects. Interior of gland dominated by



Figures 1–3. Illustrations of the foregut of *Ficus subintermedia*. **1.** Lateral view of head and partially everted proboscis. **2.** Introverted proboscis, esophagus and salivary glands, with proximal proboscis wall dissected open to show pseudo dorsal folds. **3.** Dorsal body wall dissected open to show foregut and partially everted proboscis, with anterior insertion of proboscis retractor muscles indicated by large arrow and position of buccal mass indicated by dashed arrow. Abbreviations: **bm,** buccal mass; **con,** circumesophageal nerve ring; **ey,** eye; **ja,** jaws; **ne,** neck; **oe,** esophagus; **og,** esophageal gland; **pb,** proboscis; **pf,** proboscis fold; **pn,** proboscis nerve; **prm,** proboscis retractor muscle; **rh,** rhynchodeum; **sg,** salivary gland; **sgd,** salivary gland duct; **te,** cephalic tentacle. Scale bars = 2 mm.

open lumen (Figure 15, **lu**). Branched folds of tissue, derived from gland walls, protrude into lumen (Figure 15, **se**). Epithelium lining of esophageal gland not tall or brightly stained, cells do not appear to contain obvious proteinaceous secretions. Ventral wall of esophageal gland distinguishable only as region with relatively few branching folds (Figure 15). Esophageal gland not separated from esophagus, lacking identifiable dorsal folds in this region or in posterior esophagus.

Salivary Glands: Pair of small salivary glands, connected to buccal mass by very long ducts (Figure 2, **sg**, **sgd**); composed of two equally sized lobes joined by continuous lumen (Figures 16, 17). Interior of glands convoluted, tubular pockets, each lined by small secretory cells containing large, darkly stained nuclei (Figure 17). No histological differences between anterior and posterior lobes of salivary glands. Pair of narrow salivary gland ducts pass through nerve ring with esophagus, anterior blood vessel (Figure 14), continued anteriorly along lateral surfaces of esophagus, sheathed by branches of proboscis retractor muscles (see above) (Figure 6). Salivary gland ducts insert into dorsal wall of middle part of buccal mass. Anterior section of salivary gland ducts covered by external layer of longitudinal muscle, but not fused to lateral esophageal walls (Figure 14).

DISCUSSION

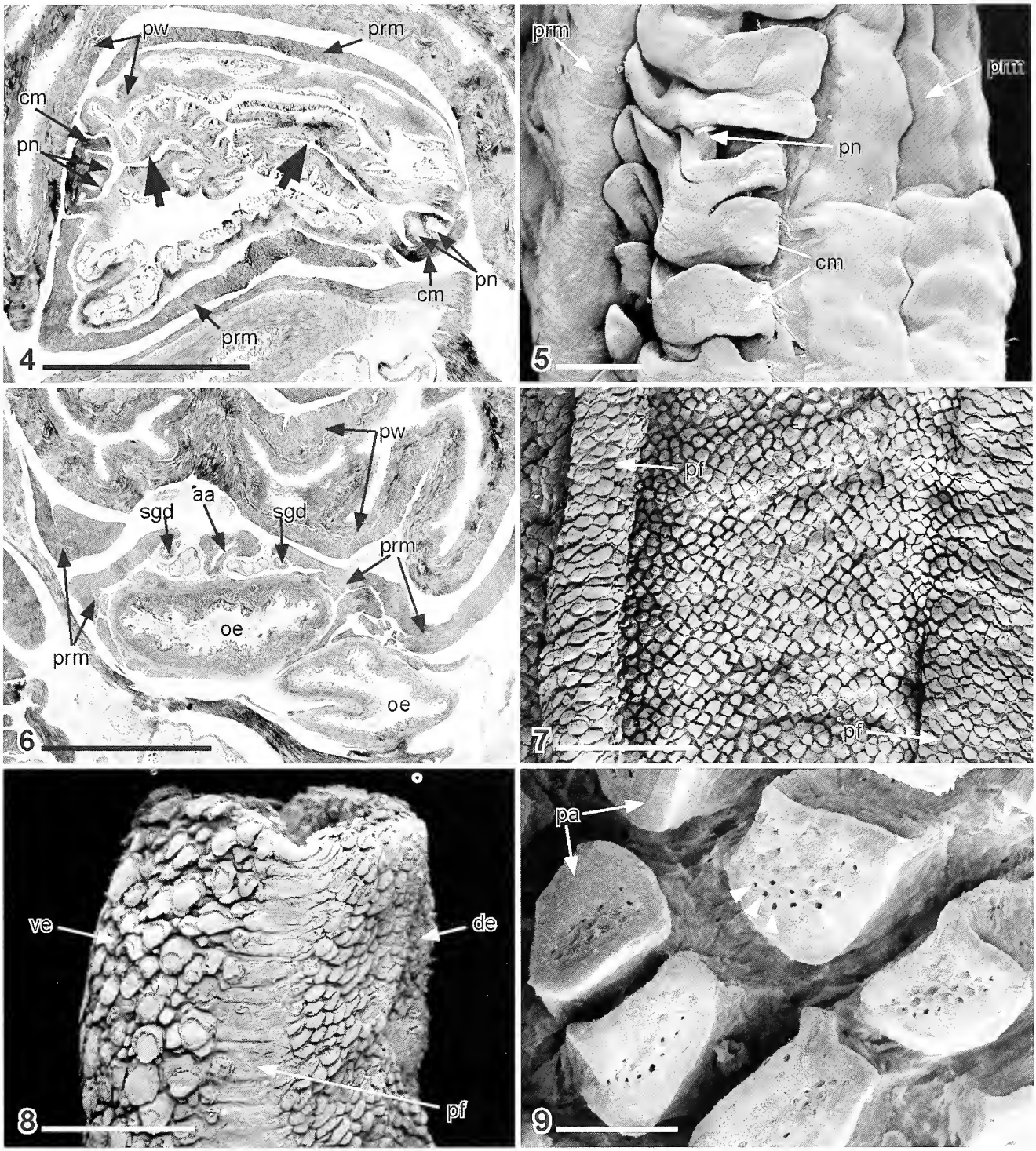
Configuration of the Ficid Foregut: The arrangement of the proboscis, retractor muscles, buccal mass and esophagus of *Ficus subintermedia*, and possibly other Ficidae, is unique in Caenogastropoda and is not shared with any other proboscis-bearing group. The extremely long ficid proboscis superficially resembles the equally long proboscis of personids such as *Distorsio* (Lewis, 1972). However, the foregut morphology of Personidae is tonnoidean (with the exception of the lack of acid-secreting proboscis glands). The proboscis of *Distorsio* is not acrembolic when introverted, but is instead retracted (i.e., not turned inside out) and coiled within the rhynchodeum (Lewis, 1972) in a fashion similar to that described for the species of the ranellid *Argobuccinum* (Day, 1969).

Although the introverted ficid proboscis is acrembolic, it is twice the length of the esophagus (Figures 2, 3), which places a physical limitation on the distance that the buccal mass can be everted anteriorly. A simple calculation of the relative lengths (excluding the elastic properties of the esophageal and proboscis walls) suggests that the buccal mass cannot be protruded beyond the level of the rhynchodeum and almost certainly cannot extend to the tip of the everted proboscis as hypothesized by Riedel (1994) (Figures 18, 19). The everted ficid proboscis appears to form a double-walled tube which funnels ingested material toward the buccal mass positioned at its base, with the proximal half of the proboscis effectively an elongated oral tube (Figure 18). This hypothesis requires confirmation through observation of the feeding behaviour of living ficids, as the mechanism or mechanisms for prey capture in this group are unclear.

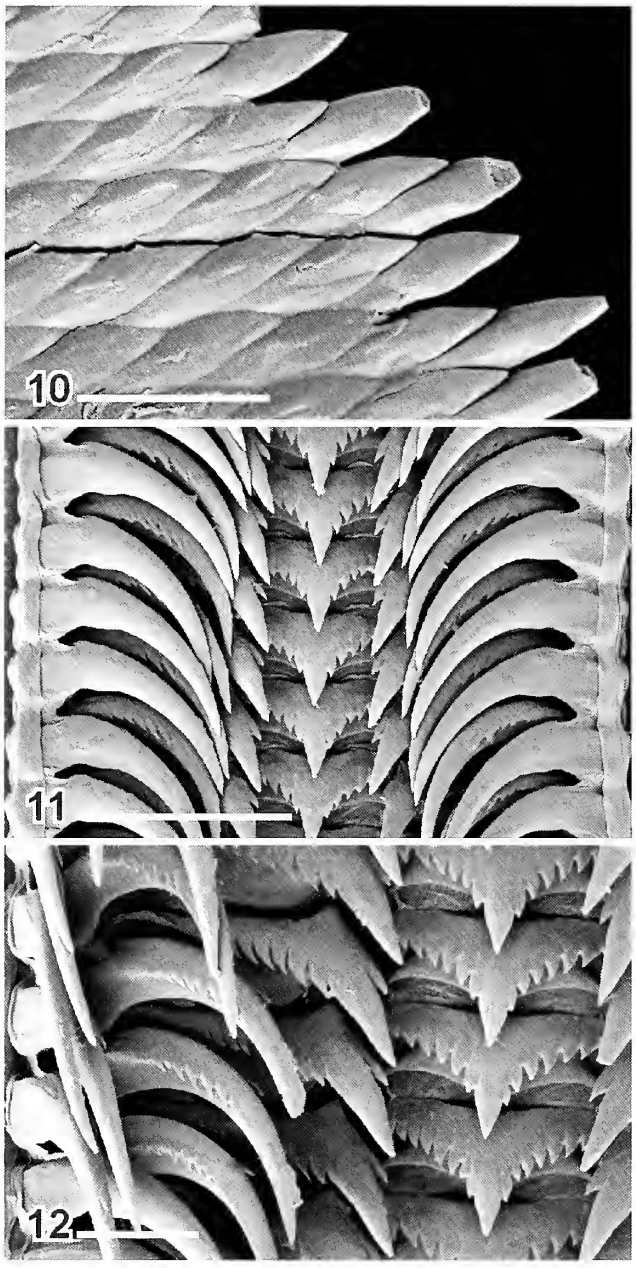
A feature supporting the interpretation of proboscis operation outlined above is the longitudinal folds that line the interior of the introverted proboscis. These proboscis folds bear a structural resemblance to the esophageal dorsal folds found in the many caenogastropods including tonnoids and most neogastropods (Graham, 1941; Strong, 2003; Andrews and Thorogood, 2005), but which were absent in the esophagus of *Ficus subintermedia*. Unlike the esophageal dorsal folds, the ficid proboscis folds are temporary and double-walled. Their presence is conditional on the introversion of the proboscis. When the proboscis is everted and the circumference increases, the folds are flattened, which prevents the appearance of the folds on the exterior of the proboscis (Figure 8). Although the position of the ficid proboscis clearly indicates that they are not homologous to esophageal dorsal folds, their convergent evolution suggests that separation of dorsal and ventral lumens confers a strong advantage for the movement of food through the digestive tract. The peculiar papillose epithelium lining the introverted proboscis is dotted with pores which suggest an excretory or absorptive function.

In the scenario described above, the buccal mass is positioned at the base of the proboscis temporarily during feeding (Figure 18). This arrangement superficially resembles some conoideans, which have a buccal mass fixed at the proboscis base—a defining feature of Conoidea which is present in all basal taxa (Taylor et al., 1993) (Figure 22). The highly unusual connection between the proboscis retractor muscles and the esophagus/buccal mass of *Ficus subintermedia* is also found in some conoideans, such as the Terebridae (Simone, 1999) (Figure 22). This evidence is insufficient to conclude homology of the ficid proboscis with the intraembolic proboscis found in some conoidean groups, but it may illustrate a path through which the intraembolic proboscis could have evolved. Retention of the buccal mass at the base of the proboscis during feeding may represent an intermediate step between an acrembolic proboscis and the permanent fixture of the buccal mass at the proboscis base (intraembolic). An alternative derivation of the intraembolic proboscis from the pleuremolic form, widely occurring in Muricoidea and Cancellarioidea, was presented by Simone (1999, fig. 27), who showed that the intraembolic proboscis is an elongation of the buccal region. These conflicting theories could be resolved by the development of a robust phylogeny of Neogastropoda.

Relationship of Ficidae to Tonnoidea and Neogastropoda: Riedel (1994) listed four morphological features shared by Ficidae and Neogastropoda. The egg mass and the configuration of the nervous system were not addressed in this study, but states of the radula and



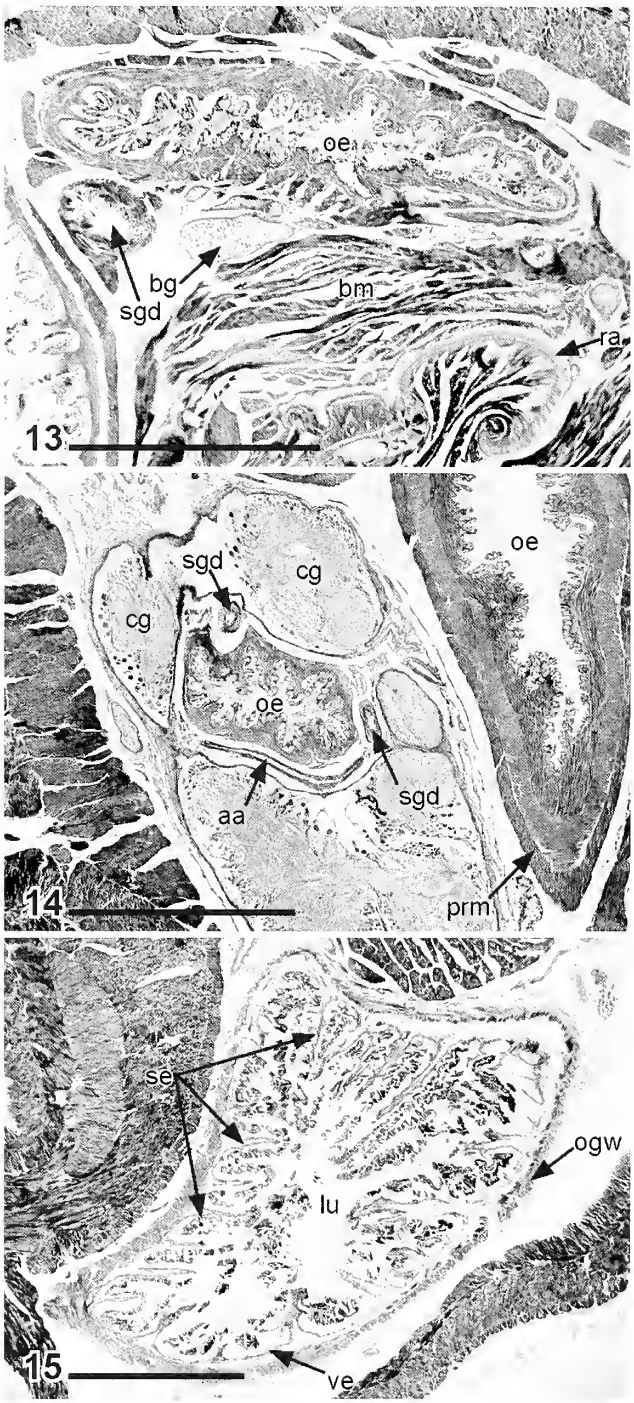
Figures 4–9. Proboscis of *Ficus subintermedia*. **4.** Transverse histologieal section through introverted proboscis, note pseudo dorsal folds formed by proboscis wall (large arrows). **5.** SEM image of exterior wall of introverted proboscis. **6.** Transverse histologieal section through haemocoel anterior to nerve ring, with proboscis retractor muscles attaching to esophagus. **7.** SEM image of interior wall of introverted proboscis, dissected by longitudinal incision in ventral surface, showing papillose surface. **8.** SEM image of lateral exterior wall of everted proboscis tip. **9.** SEM image showing detail of epithelium lining proboscis wall, pores in papilla (**pa**) are marked with white triangles. Abbreviations: **aa,** anterior aorta; **cm,** circular muscle; **de,** dorsal epithelium; **oe,** esophagus; **pa,** papilla; **pf,** proboscis fold; **pn,** proboscis nerve; **prm,** proboscis retractor muscle; **pw,** proboscis wall; **sgd,** salivary gland duct; **ve,** ventral epithelium. Scale bars: Figures 4, 6–8 = 1 mm; Figure 5 = 250 μm; FIGURE 9 = 50 μm.



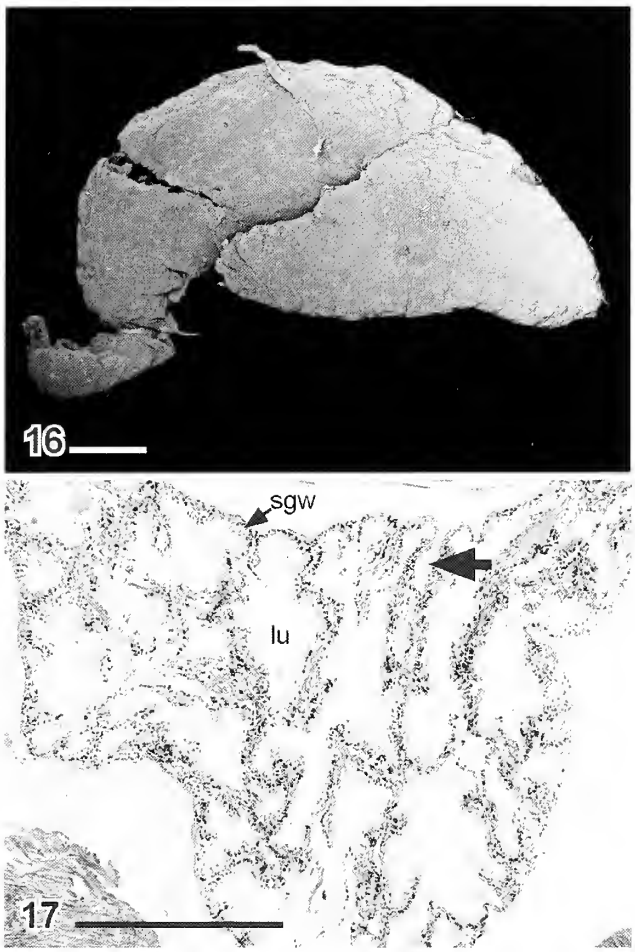
Figures 10–12. SEM images of the jaws and radula of *Ficus subintermedia*. **10.** Detail of jaw composed of rods. **11.** Radula. **12.** Detail of radular teeth. Seale bars: Figures 10, 12 = 100 μm; Figure 11 = 250 μm.

proboscis can be reassessed as potential synapomorphies. Densely-packed teeth on the fieid radula were postulated as an intermediate between the taenioglossan and stenoglossan radular patterns (Riedel, 1994). However, the radular dentition of *Ficus subintermedia* is very similar to that of tonnoidean and other higher caenogastropods (Warén and Bouchet, 1990; pers. observ.) and is not remarkable (Table 1).

The introversion (turning inside out) of the proboscis was correctly identified by Riedel (1994) as a character differentiating Tonnoidea and Fieoidea, as the tonnoid



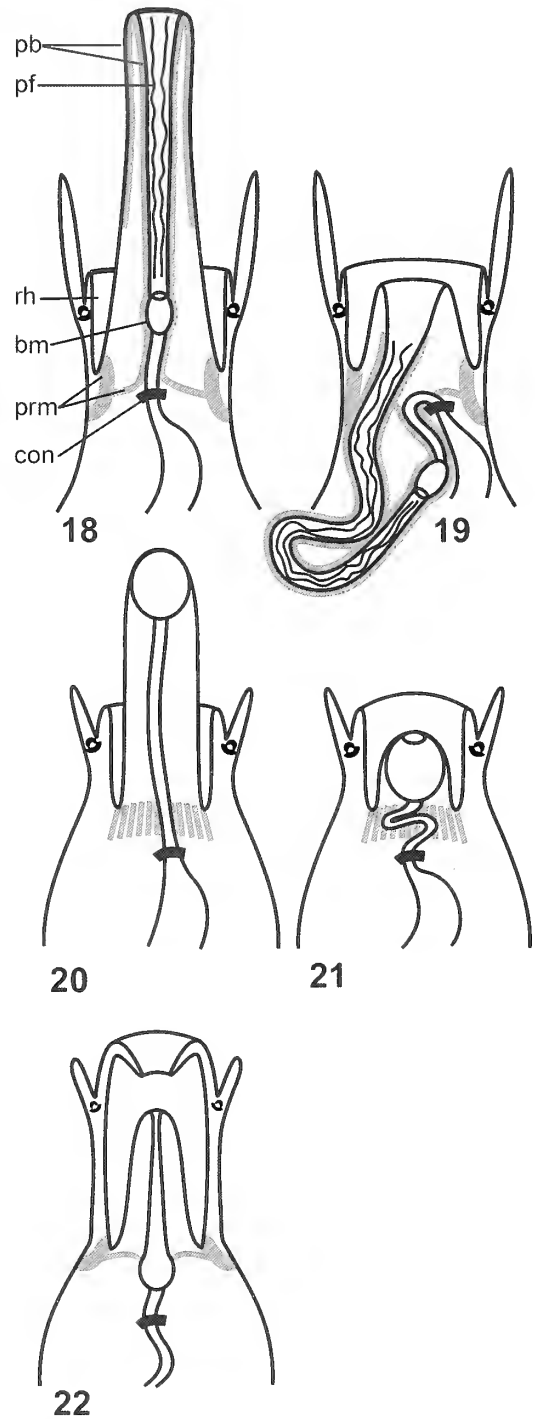
Figures 13–15. Histological seetions through the esophagus of *Ficus subintermedia*. **13.** Oblique section through anterior esophagus adjaeent to bueeal mass. **14.** Transverse section through esophagus and eireumesophageal nerve ring. **15.** Transverse section through esophageal gland. Abbreviations: **aa,** anterior aorta; **bg,** buccal ganglia; **bm,** buceal mass; **cg,** cerebral ganglion; **lu,** lumen; **oe,** esophagus; **ogw,** esophageal gland wall; **prm,** proboscis retractor muscle; **ra,** radula; **se,** septum; **sgd,** salivary gland duet; **ve,** ventral epithelium. Seale bars = 1 mm.



Figures 16–17. Salivary gland of *Ficus subintermedia*. **16.** SEM image of bilobed salivary gland. **17.** Histological section through salivary gland, note incomplete separation between lobes marked with a large arrow. Abbreviations: **lu,** lumen; **sgw,** salivary gland wall. Scale bars = 500 m.

proboscis is retractile but can not truly be introverted (Day, 1969; Simone, 1995) (Figures 20, 21, Table 1). However, introversion is a feature of the acrembolic proboscis of several other distantly related caenogastropod groups (including naticoids and ptenoglossans) as well as the pleurembolic proboscis of Neogastropoda, and could not alone be considered a potential synapomorphy. The superficial similarities between the ficid and conoidean proboscis, discussed above, are inconclusive.

A comparison between Ficidae, Tonnoidea, and Neogastropoda shows that there are few potential synapomorphies (Table 1). A pair of dorsal jaws, composed of rods, is present at the anterior margin of the buccal mass of *Ficus subintermedia*. These are alike in position and composition to those of *Tonna galea* (Weber, 1927) and most other middle caenogastropods (Strong, 2003), while paired jaws are not present in neogastropods (Strong, 2003). But as jaws are plesiomorphic in Caenogastropoda, they are not informative in assessing the monophyly of Ficidae with Tonnoidea or Neogastropoda.



Figures 18–22. Diagrammatic representations of proboscis configuration, with proboscis retractor muscles shaded grey. Salivary glands are not illustrated. **18, 19.** *Ficus subintermedia* **18.** Proboscis everted. **19.** Proboscis introverted. **20, 21.** A tonnoidean, modified from Day (1969). **20.** Proboscis everted. **21.** Proboscis introverted. **22.** Terebridae (Conoidea), with intraembolic proboscis, modified from Simone (1999: fig. 27). Abbreviations: **bm,** buccal mass; **con,** circumesophageal nerve ring; **pb,** proboscis; **pf,** proboscis fold; **prm,** proboscis retractor muscle; **rh,** rhynchodeum. Not to scale.

Table 1. A comparison of the main features of the proboscis and foregut of Ficidae, Tonnoidea and Conoidea, using information available in the literature (see text for references).

Foregut Anatomy	Ficidae	Tonnoidea	Conoidea
Proboscis	Very long, acrembolic	Moderately long, contractile, not introvertable	Various lengths and forms, including intraembolic, pleurembolic, reduced/absent
Proboscis lumen (when introverted)	With pseudo dorsal folds	Not applicable as proboscis does not introvert	Simple
Proboscis retractor muscles	Attaching to proximal proboscis wall and esophagus, sheathing anterior esophagus, buccal mass and distal proboscis	Short, attaching to proximal proboscis wall	Attaching to interior of proboscis wall, also attaching to buccal mass in some taxa (Terebridae)
Permanent (external) rhynchodeum	Present	Present	Present, introvertable in some taxa
Buccal mass	Small	Large	Variable, reduced/absent in some taxa
Jaws	Paired dorsal jaws present, composed of rods	Paired dorsal jaws present, composed of rods	Jaws absent
Radula	Taenioglossan	Taenioglossan	Variable, 5 or fewer teeth, absent in some taxa
Salivary glands	Single pair of small salivary glands; bilobed, homogeneous	Single pair of salivary glands plus proboscis glands derived from salivary glands (except Personidae)	Salivary glands usually present, accessory salivary glands present in some taxa
Anterior esophagus	Dorsal and ventrolateral folds absent	Prominent dorsal and ventrolateral folds present	Ventrolateral folds absent, dorsal folds reduced or absent
Esophageal gland	Open lumen with few septate folds, low glandular epithelium, confluent with esophagus	Dense septate folds, tall glandular epithelium, confluent with esophagus	Tubular venom gland, with tall glandular epithelium and muscular bulb

Salivary gland form varies considerably between caenogastropods, with a pair of accessory salivary glands present in many neogastropods and an extremely large pair of acid-secreting proboscis glands, derived from the salivary glands, present in tonnoids (except Personidae) (Weber, 1927; Simone, 1995; Andrews et al., 1999) (Table 1). The anterior (acinous) and posterior (acid-secreting, proboscis) salivary glands of *Cymatium intermedius* have distinct histologies reflecting their specialized functions (Andrews et al., 1999). Although the salivary glands of *F. subintermedia* are superficially bilobed, the histology is homogeneous. The salivary glands of tonnoideans and other caenogastropods are typically composed of large cells with narrow lumens (Andrews et al., 1999). The salivary glands of *F. subintermedia* are unusual in that they are dominated by an expanded lumen, perhaps for storing saliva. The absence of either accessory salivary glands or proboscis glands is uninformative in establishing the relationship of *Ficus* to either Tonnoidea or Neogastropoda.

Modification of the esophageal gland to form a discrete organ, the gland of Leiblein or its partial homologue the venom gland (Ponder, 1970), is a feature common to most neogastropods. Unlike most other caenogastropods including Tonnoidea, the esophageal gland of *Ficus subintermedia* was poorly developed and formed a sac-like expansion of the esophagus (Table 1). The digestive properties of the ficid esophageal gland are entirely unknown.

Ficids display a variety of morphological synapomorphies which, at this stage of our knowledge of caenogastropod anatomy, confound attempts to affiliate the group with other higher caenogastropods. Some aspects of proboscis morphology, together with the simplified esophagus and reduced buccal mass, could be seen as suggesting an association with Neogastropoda. Comparisons between taxa are helpful for elucidating homology, but the phylogenetic affinities of Ficidae require further investigation using cladistic methodology, and given their unusual morphology, with a particular focus on molecular data.

ACKNOWLEDGMENTS

Drs. M.G. Harasewych and E.E. Strong of the Smithsonian Institution are thanked for organizing the neogastropod symposium at the 2007 World Congress of Malacology where this research was first presented. Sue Lindsay at the Australian Museum SEM facilities kindly assisted with specimen preparation and SEM operation. This project was funded by an Australian Biodiversity Resources Study Postgraduate Research Scholarship and a Chadwick Biodiversity Fellowship at

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Book Review

Marine Shells of Northeast Florida

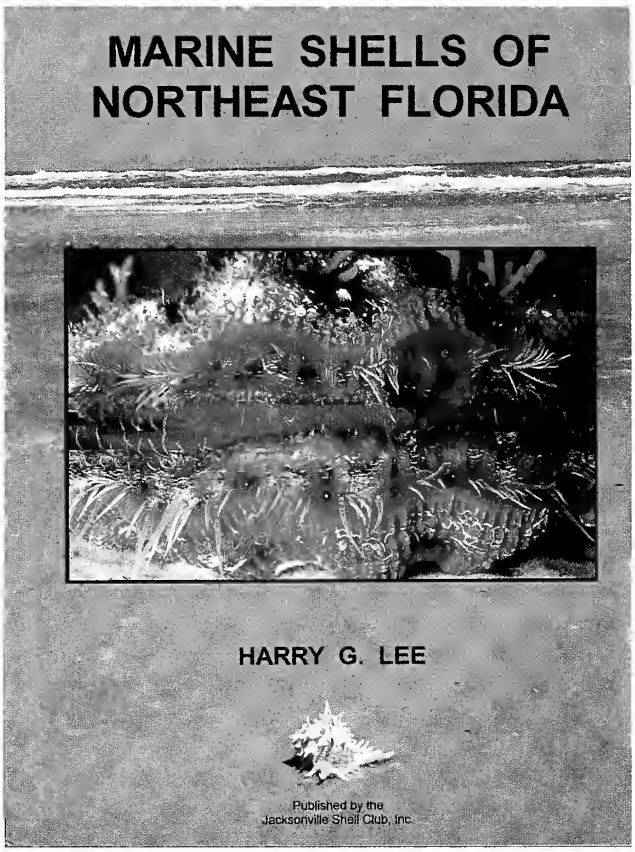
Lee, H.G. 2009. Marine Shells of Northeast Florida. Jacksonville Shell Club, Jacksonville, 204 pp., 19 color pls. nflshells@bellsouth.net, http://www.jaxshells.org.

In 1975, William G. Lyons, the well-known research malacologist and former senior administrator of the Florida Marine Research Institute in St. Petersburg Beach, Florida, provided the inspiration that began the project resulting in *Marine Shells of Northeast Florida*. Mr. Lyons, in an article in *Shell-O-Gram*, the publication of the Jacksonville Shell Club, noted that information on seashells of the northeastern coast of Florida was probably less available than for any other section of the state, and suggested that the Jacksonville Shell Club had the expertise to solve the problem. Under his capable hands, vast experience, intellectual know-how, and hard work, Dr. Harry Lee has led the Club to complete the challenge set by Mr. Lyons. They have achieved their goal *summa cum laude*.

The work is organized into a dedication, a preface, a table of contents, an introduction, materials and methods, a map of the area treated (with a legend depicting important landmarks and collecting stations), abbreviations for private and institutional repositories, inspirational quotations from several famous naturalists, taxonomic treatment, discussions and conclusions, acknowledgements, literature cited, and index.

Introductory comments state that the geographic boundaries of this study cover the estuarine and marine waters extending from Nassau, Duval, and St. Johns Counties eastward to near the edge of the Continental Slope (circa 55 m). A quick geological note and more extensive ecological observations of the area in question are followed by a review of the publications dealing with Florida's malacofauna.

In the Materials and Methods section the author seeks to impress upon the reader the collaborative efforts made for this project by no less than 63 individuals, in an expanse of time of some 34 years, and at more than 100 stations. This collaborative effort is emphasized by the use by of the first person plural "we" and "our" throughout the text. It is also reflected in the long list of acknowledgements at the end of the book. As expected from material collected by so many people for so many years, the list of collecting techniques is long and varied, from beachcombing to dredging, and from clam-raking to the analysis of gut contents of malacophagous marine creatures. Identification of species was made by using standard books and periodicals in the field, Malacolog 4.1.0 (Rosenberg, 2005), and consultation with specialists. Almost all material presented was directly examined and identified by the author.



The taxonomic report lists the taxa according to contemporary arrangement, that is, at the family level and above in phylogenetic sequence, with genera and species following in alphabetical order. Species-level taxa are sequentially numbered. The official vernacular name (Turgeon et al., 1998) accompanies each species; where no official vernacular name was available, one was created. The vernacular name is followed by a bracketed number that indicates the frequency of occurrence of the species, and this in turn is followed by the maximum size recorded for the species collected in the course of the study. Many of these maximum sizes are larger than those published in world-records publications (e.g., Hutsell et al., 2001); if the species is not available in those publications, it is compared with Abbott's (1974) maximum stated size. No species-size bias was detected in this work, where such taxa as a 1 mm *Didianema* sp. and a 460 mm *Triplofusus giganteus* are represented. Special attention is given to the occurrence of sinistrality in a species.

The next entry is a listing of locality data, in bathymetric order, for the occurrence of the taxon being treated; data generally include depth, substrate, method of collection,

collector, and repository. A halftone image of the species aeeompanies the description in most instances. Whenever possible, an authentie northeast Florida speeimen was figured. The final section of eaeh of the species treated deals with comments by the author, which may include eeological, behavioral, nomenclatorial, taxonomie, or geographic perspeetives. Emphasis is placed on species deseribed after Abbott's (l974) publication.

The taxonomic section comprises 147 pages; although the last species treated is number 798, there are six last-minute entries for a total of 804 speeies. This section includes 2 speeies in the class Polyplacophora, 232 in Peleeypoda, 10 in Scaphopoda, 551 in Gastropoda, and 9 in Cephalopoda. Besides meticulous loeality data, depth, substrate, and method of collection, in many instances there are additional data that eontribute to a better understanding of the ecologieal and biologieal contexts of the speeies (e.g., ex heart urchin (*Meoma v. ventricosa);* ex-seastar (*Astropeeten artieulatus*); ex-batfish). Speeialists in the field have been consulted for the proper identification of the host species. Many of the listed taxa (approximately 10%) are either undescribed or a specifie epithet eould not be applied to it, while others had not been reeorded by some of the more reeent publications (e.g., Camp et al., 1998; Turgeon et al., 1998).

Well-known speeies may laek "random eomments," or they may have only a brief comment on geographieal extension. These extensions usually refer to Abbott (1974), although many have already been reported by Rosenberg (2005). However, the years of eareful researeh by the author make this seetion the heart and soul of the book. The more obscure the taxon and the more complicated a species-eomplex may be, the longer the comments are. Some of the more elucidating treatments are in members of the more cryptie families sueh as Cerithiopsidae, Triphoridae, Caeeidae, and Eulimidae; some of the better treated genera are *Turbonilla* and *Olivella*. The comments deal with eomparisons of the speeies with congeners, pseudo-congeners, Recent and fossil species, western Atlantie, eastern Atlantic, and even Panamic Provinee taxa. The possibility of synonymy with other species, the possibility of a complex of species within a taxon (e.g, *Ctena orbieulata* Montagu, 1808), errors in authorship (e.g., *Seaphella junonia* Shaw, 1808, instead of Lamarck, 1804), errors in dates, ete., are only some of the information that one may encounter in this section. Some taxa are treated conservatively (e.g., *Strombus eostatus* rather than *Aliger eostatus*), and many readers may agree with this assignment; others follow some of the latest research (e.g., *Cryoturris*, *Daphnella*, *Ithyeythara*, etc., placed in Conidae), and many readers will disagree with the assignment.

Although most of the speeies are accompanied by an image, these are of low definition and small, perhaps no more than one square inch. In most eases the images by themselves will not serve as a means of positive identification of the species; however, this drawbaek is overcome by the careful comparison of the species with similar taxa, by references to high definition images of the species in other publieations (usually Gundersen, 1998), and by 19 color plates depicting the more commonly eneountered species (including some living mollusks). Moreover, excellent images of many of the species shown in the book may be seen at http://www.jaxshells.org/marine.htm.

The eareful research that culminated in the plethora of information provided in the comments is reflected in the 17 pages of "Literature Cited," which lists some 400 references, many of which had long been forgotten or ignored until now.

Few problems showed up in my reading of the text, mostly trivial "typos" easily overlooked. A *lapsus mentis* occurred when, in the comments on species No. 548, there appears the name *Costoanaehis lafresnayi* instead of *C. translirata*. Also, a grammatical error was noted (*Epitonium echinaticostum* for *E. echinaticosta*), and two references were missing from the literature cited, those of *Agathotoma ecthymata* García, 2008a and *Anna florida* García, 2008b).These omissions are understandable as the two taxa were last-minute additions to the ms.

Marine Shells of Northeast Florida is the essence of what a regional faunal treatment should be. It is exhaustive in the treatment of species, and is accurately and meticulously documented and researched in all aspects. But this publication deals with much more than the regional fauna, and it will prove to be of immense value to the malacologist, the amateur shell colleetor, and to researchers in related fields with interest not only on the marine malacofauna of northeastern Florida, but of the entire western Atlantic. Let us hope that future malacological books follow the 21st Century approach of this publication and its worthy companions *Bahamian Seashells* (Redfern, 2001) and *South Florida Seashells* (Mikkelsen and Bieler, 2000).

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Notice

Florida United Malacologists (FUM): First Announcement

The first meeting of the Florida United Malacologists (FUM) will occur Saturday, January 30, 2010, at The Bailey-Matthews Shell Museum (BMSM) in Sanibel, Florida. The one-day gathering is designed to enhance communication among professional, amateur, and student malacologists, with topics including but not limited to biology, ecology, paleontology, archaeology, and conservation.

FUM follows the pattern established by similar informal gatherings such as BAM (Bay Area Malacologists), SCUM (Southern California United Malacologists), MAM (Mid-Atlantic Malacologists), and OVUM (Ohio (River) Valley United Malacologists). There is no formal membership, dues, officers, nor publications. However, submission of brief abstracts is required. Abstracts, limited to 150 words or less, will be posted on the Museum web site. The gathering will be free of charge to presenters and Museum members. Non-members will be asked to donate the Museum admission fee of $7. Participants are strongly encouraged to ask questions and discuss data, compare notes on methods and problems, and get acquainted with presenters and members of the audience. Presentations, limited to 15 min+5 min for questions, will be informal and will cover current research, collecting efforts, and collection issues. The Museum will provide projection equipment for PowerPoint programs, brief videos, and slides.

Due to staffing limitations, use of the library and research area and collection visits will be limited to two days prior to the gathering, Thursday, January 28, and Friday, January 29. Museum parking is free. Box lunches and dinner at a local restaurant will be available at cost to participants and presenters. A reservation form for participation in the event (for presenters and participants) will soon be posted in the Museum web site (www.shellmuseum.org). Seating is limited, so please return the reservation form prior to November 30, 2009.

Please send all inquiries, reservations, and submission of presentation topics to Dr. José H. Leal at jleal@shellmuseum.org. The deadline for submission of topics and abstracts will be December 15, 2009. The FUM program with abstracts, times, and sequence of presentations will be posted on the Museum web site, www.shellmuseum.org, shortly after the deadline for submission of topics.

Sponsored in part by the State of Florida, Department of State, Division of Cultural Affairs, the Florida Arts Council and the National Endowment for the Arts.

NATIONAL
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INSTRUCTIONS TO AUTHORS

THE NAUTILUS publishes articles on all aspects of the biology, paleontology, and systematics of mollusks. Manuscripts describing original, unpublished research and review articles will be considered. Brief articles, not exceeding 1000 words, will be published as notes and do not require an abstract. Notices of interest to the malacological community will appear in a notices section.

Manuscripts: Each original manuscript and accompanying illustrations should be submitted to the editor preferably via e-mail or as hardcopy in triplicate.

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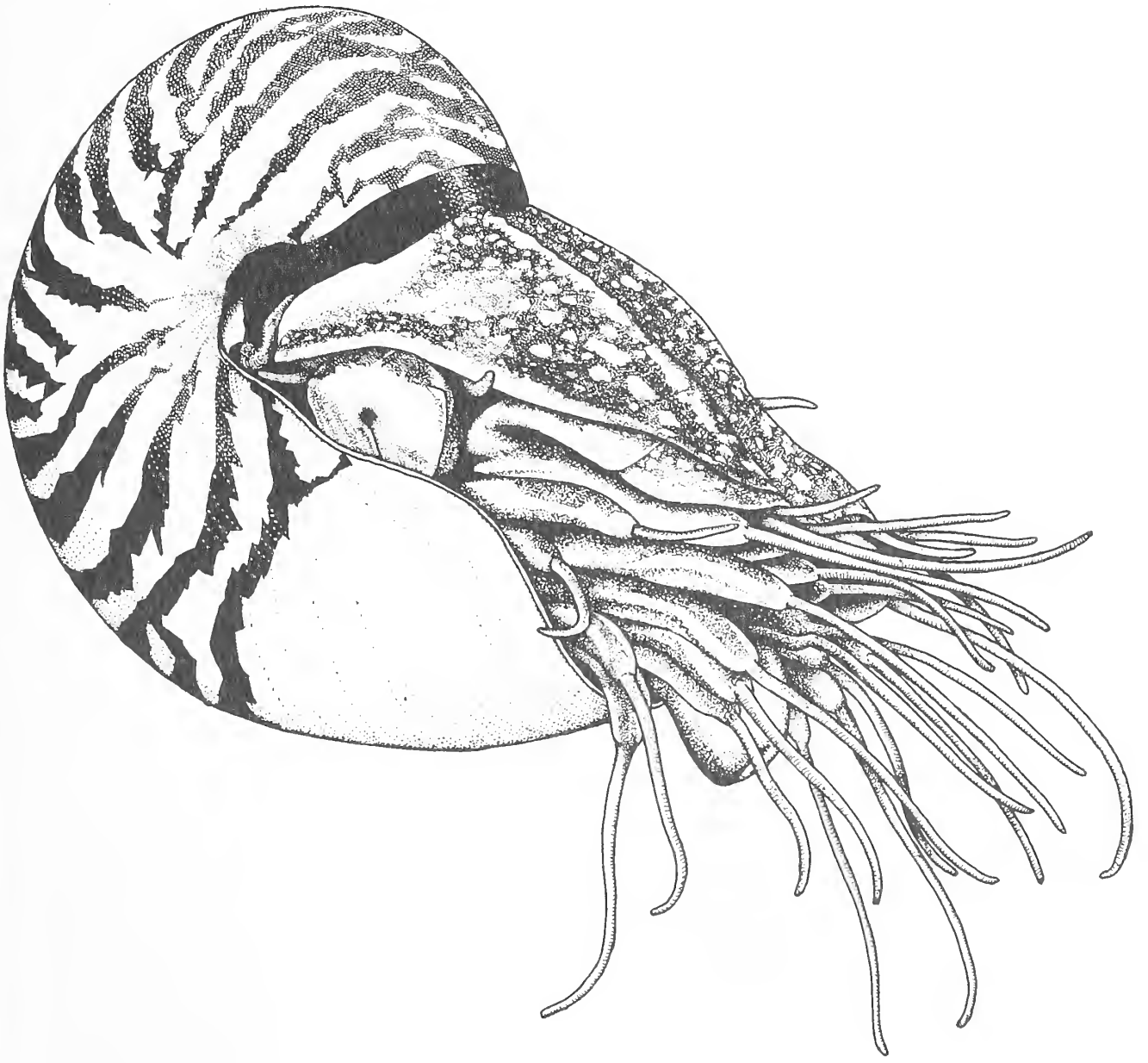

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ABSTRACT

The western Atlantic members of the genera *Anna* Risso, 1826, *Antillophos* Woodring, 1928, *Bailya* M. Smith, 1944, *Caducifer* Dall, 1904, *Cumia* Bivona-Bernardi, 1838, *Monostiolum* Dall, 1904, and *Parviphos* Sarasua, 1984, and a new genus, *Dianthiphos*, are reviewed. The following taxa are recognized for *Anna*: *A. florida* García, 2008, *A. milleri* (Usticke, 1959), and *A. willemsae* (De Jong and Coomans, 1988). *Anna royalensis* new species is described from Roatán and Utila Islands, Honduras. The following taxa are recognized for *Antillophos*: *A. bahamasensis* Petuch, 2002, *A. beauii* (Fischer and Bernardi, 1857), *A. candeanus* (d'Orbigny, 1842), *A. chazaliei* (Dautzenberg, 1900), *A. oxyglyptus* Dall and Simpson, 1901, *A. smithi* (Watson, 1885), and *A. virginiae* (Schwengel, 1942). *Antillophos verriculum* new species is described from the Guajira Peninsula, Colombia. The following taxa are recognized for *Bailya*: *B. intricata* (Dall, 1884), *B. parva* (Adams, 1850), and *B. weberi* (Watters, 1983). *Bailya morgani* new species is described from Roatán Island, Honduras, and *Bailya sanctorum* new species is described from St. Thomas, US Virgin Islands. The following taxon is recognized for *Caducifer*: *C. atlanticus* Coelho, Matthews, and Cardoso, 1970. *Caducifer camelopardalus* new species is described from Bahia State, Brazil. The following taxon is recognized for *Cumia*: *C. sunderlandi* (Petuch, 1995). *Cumia clavula* new species is described from Costa Rica. The following taxa are recognized for *Monostiolum*: *M. auratum* Watters and Finlay, 1989, *M. harryleei* García, 2006, *M. tessellatum* (Reeve, 1844), and *M. rosewateri* Watters and Finlay, 1989. *Monostiolum nocturnum* new species is described from Tobago and *Monostiolum fumosum* new species is described from Isla Coche, Venezuela. The following taxa are recognized for *Parviphos*: *P. adelus* (Schwengel, 1942) and *P. marijkae* (De Jong and Coomans, 1988). *Parviphos chalcedonius* new species is described from the Mariel sands, Cuba. The genus *Dianthiphos* new genus is described, with *D. bernardoi* (Costa and Gomes, 1998) as its type species. *Dianthiphos electrum* new species is described from the Guajira Peninsula, Colombia. *Engina goncalvesi* Coltro, 2005, is compared with species of *Parviphos*. *Hesperisternia itzamnai* new species is described from Yucatan, Mexico.

INTRODUCTION

Many genera of small buccinids from the western Atlantic Ocean have not been comprehensively reviewed since Tryon (1881). Since that time numerous species have been described as the results of trawling, dredging, and SCUBA collecting in previously inaccessible locations. In particular, an abundance of material has been brought to light by commercial collectors. Much of this material has yet to make its way into institutional collections. It has become apparent that the discovery of new taxa has outpaced their description and that the identification of even the most commonly encountered species has become problematic. To that end, the western Atlantic members of the genera *Anna* Risso, 1826, *Antillophos* Woodring, 1928, *Bailya* M. Smith, 1944, *Caducifer* Dall, 1904, *Monostiolum* Dall, 1904, and *Parviphos* Sarasua, 1984, and a new genus, *Dianthiphos*, are reviewed here. The western Atlantic species of the colubrariid genus *Cumia* Bivona-Bernardi, 1838, are also reviewed.

With the exception of *Anna* and *Caducifer*, these genera appear to be of New World origin. In the eastern Pacific Ocean *Antillophos* is represented by *A. veraguensis* (Hinds, 1843), a cognate of the western Atlantic *A. virginiae* (Schwengel, 1942). *Bailya* is present there as *Bailya anomala* (Hinds, 1844). *Monostiolum* occurs as *M. crebistriatus* (Carpenter, 1856) (the cognate of the Pliocene Floridian species *M. thomasi* (Olsson, 1967) and *M. petiti* (Olsson, 1967)) and *M. pictum* (Reeve, 1844). *Parviphos* is represented by *P. nigricostatus*

(Reeve, 1846) (previously regarded as a *Monostiolum*). On the other hand, no members of *Antillophos*, *Bailya*, *Cadnicifer*, *Monostiolum*, *Parviphos*, or *Dianthiphos* have been recorded from the eastern Atlantic Ocean (Ardovini and Cossignani, 2004), although *Anna* is represented by *A. assimilis* (Reeve, 1846).

Based on a phylogenetic study using shell morphology, Haasl (2000) suggested that the Photinae Gray, 1857 (considered synonymous with Pisaniinae Gray, 1857, by Bouchet and Rocroi, 2005, and including many of the genera discussed in this study) was sister group to his Nassariinae Iredale, 1916, both having been derived from the American Eocene-Oligocene *Tritiaria*. This considerably confounds the limits between the Buccinidae and Nassariidae (the latter considered paraphyletic by Haasl, 2000) and the correct placement of the pisaniines. Ponder and Warén (1988) had also united the Nassariidae (among others) with the Buccinidae, but few recent authors have followed this conclusion.

MATERIALS AND METHODS

Shell length is measured from the tip of the apex to the end of the siphonal canal. Width is measured as the maximum dimension in a plane with the aperture perpendicular to the axis of coiling. Spiral sculpture is counted from the suture to the end of the siphonal canal. Axial sculpture counts refer to inter-varical sculpture; varices and any sculpture on varices are treated separately. Lirae counts within the outer lip may include bifurcating lirations. Locality information, aside from type locality designations, may have been augmented from the original label for clarification. Given the generalized nature of most label information, no attempt has been made to georeference sites that did not originally include coordinates. Dimensions in captions refer to shell length.

The primary collections used for this study were The Bailey-Matthews Shell Museum, Sanibel, FL, USA and the Florida Museum of Natural History, Gainesville, FL, USA, with material from the collections of Colin Redfern, Boca Raton, FL, USA, Emilio F. García, Lafayette, LA, USA, Harry G. Lee, Jacksonville, FL, USA, and the author's collection. Additional material was provided by the Academy of Natural Sciences, Philadelphia, PA, USA, the Field Museum of Natural History, Chicago, IL, USA, the Muséum national d'Histoire naturelle, Paris, France, the Natural History Museum, London, UK, the Ohio State University Museum of Biological Diversity, Columbus, OH, USA, the U.S. National Museum of Natural History, Washington D.C., USA, and the Zoölogisch Museum, Amsterdam, The Netherlands.

Abbreviations used in the text are: AMNH: American Museum of Natural History, New York City, NY, USA; ANSP: Academy of Natural Sciences, Philadelphia, PA, USA; BM(NH): Natural History Museum, London, UK; BMSM: The Bailey-Matthews Shell Museum, Sanibel, FL, USA; CR: collection of Colin Redfern, Boca Raton, FL, USA; EFG: Collection of Emilio F. García, Lafayette, LA, USA; FMNH: Field Museum of Natural History, Chicago, IL, USA; GTW: Collection of the author, Columbus, OH, USA; HGL: Collection of Harry G. Lee, Jacksonville, FL, USA; MCZ: Museum of Comparative Zoology, Cambridge, MA, USA: MNHN: Muséum national d'Histoire naturelle, Paris, France; OSUM: Ohio State University Museum of Biological Diversity, Columbus, OH, USA; UF: Florida Museum of Natural History, Gainesville, FL, USA; USNM: U.S. National Museum of Natural History, Washington D.C., USA; ZMA: Zoölogisch Museum, Amsterdam, The Netherlands.

SYSTEMATICS

Family Buccinidae Rafinesque, 1815
Subfamily Pisaniinae Gray, 1857

Genus *Anna* Risso, 1826

Anna Risso, 1826: 214.

Type Species: *Anna massena* Risso, 1826, by monotypy.

Description: Small-sized for the family (to 27 mm, but usually $\ll$ 12 mm). Fusiform; aperture ca. 50% of shell length. Protoconch of 1.5 small, smooth, rounded whorls. Teleoconch sculpture of narrow, spiral cords and prominent axial ribs. Terminal varix thickened, wide, slightly or not at all reflected abaperturally. Aperture lirate within outer lip. Columella with denticles along much or all of its length. Columella distinctly angled at siphonal canal. See Table 1 for comparison with other genera.

Discussion: The species discussed here are assigned to *Anna* with some reservation. Vermeij (2006) did not include *A. milleri* or *A. willemsae*, or any western Atlantic species, in his list of *Anna* species (both *A. milleri* and *A. willemsae* were previously relegated to other genera). Vermeij's concept of *Anna* included shells with 11 or more axial ribs whereas only *A. florida* of the western Atlantic Ocean species has more than 10 ribs. The western Atlantic Ocean species also have long lirae within the inner lip in contrast to the much shorter lirae of other species of *Anna*. The species discussed here are congeneric but may belong to an as yet unnamed genus.

In the western Atlantic Ocean, *Anna* is most similar to *Parviphos*. *Parviphos* differs from *Anna* in being larger, less fusiform, and in the structure of its protoconch and terminal varix. The protoconch of *Parviphos* is tabulate whereas the protoconch of *Anna* is rounded. The terminal varix of *Parviphos* is massive, produced outwards, and reflected abaperturally. The terminal varix of *Anna* is also massive but does not project as far out from the whorl or reflect backward to the same degree.

Anna florida García, 2008
(Figures 1–16)

?Cantharus massena "Risso, 1826" Dall and Bartsch, 1911: 287; Abbott, 1974: 219 [? non Risso, 1826, possible misidentification].

Table 1. Shell characteristics of genera exclusive of *Engina*. * excludes denticles bordering anal or siphonal canal.

	Protoconch	Previous varices	Columella continuous or angled	Columella with dentieles*	Outer lip lirate	Decollate
Anna	rounded smooth	no	angled	yes	yes	no
Antillophos	conical keeled	yes	angled	some species	yes	no
Bailya	conical smooth	no	continuous	no	yes	no
Caducifer	tabulate smooth	no	angled	no	some species	yes
Cunia	minute, not differentiated	yes	slightly angled	no	no	no
Dianthiphos	bulbous smooth	no	angled	no	no	no
Monostiolum	tabulate smooth	no	angled	no	no	no
Parviphos	tabulate smooth	no	angled	some species	yes	no

Anna florida García, 2008a: 142–145, figs. 1–8.

Description: Average size 14.2 mm in length (min, 12.9; max, 16.2). Fusiform; spire ca. 60% total length. Protoconch small, of 1.5 smooth, white whorls with tan blotches. Teleoconch of 5.75 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 13 rounded, widely separated, spiral threads, including siphonal canal, with intercalated 2° threads or cords. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely spaced, high ribs; 9–13 ribs on penultimate whorl, ca. 13 ribs on last whorl, not including varix. Intersections of axial and spiral sculpture with strong, elongated nodules. Terminal varix well-developed, somewhat constricted, wide. Aperture oval, outer lip with 7–9 lirate teeth. Columella angled at siphonal canal, bounded by 2 plications; 3–5 minute denticles along partially erect parietal wall; one denticle bounding anal canal on columella. Siphonal canal short, open. Color white with darker orangish-tan axial ribs broken by one pale band at subperiphery and another on siphonal canal, markings aligned into flammulations or as polka dots. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: Holotype ANSP 418032.

Type Locality: 73 mi. WSW of Anna Maria Key, W. Florida, Gulf of Mexico, in 50 m.

Paratypes: ANSP 418033, 1 shell, 27°42.71′ N, 84°13.09′ W, in 68–68.5 m; EFG 25352, 1 shell, 70.6–72.9 m, 24°44.77′ N, 83°43.71′ W; EFG 13089, UF 419133, HGL, each 1 shell, 2 m, off Sugarloaf Key bridge, S Florida; HGL, 1 shell, 59–117 m (*ex-pisce*), off west Florida; HGL, 2 shells, 44–50 m, 68–83 km off Ponte Vedra, St. John's Co., Florida; USNM 1111876, HGL, each 1 shell, 0 m, Turtle Beach, south coast of Bermuda.

Other Material Examined: Florida. UF 266955, off Miami, 80 m, Miami-Dade Co; UF 154765, off Destin, 28 m, Okaloosa Co.; UF 289781, off St. Petersburg, 27°56′ N, 84° 29′ W, Pinellas Co.; UF 150206, 58 m, off Naples, 26°35′ N, Collier Co.; UF 186142, 100 m, 200° off Sand Key, Triton Sta. 956, Monroe Co.; FMNH 154784, 191310, 191364, UF 70453, all Bonefish Key, Monroe Co.

Distribution: This species is known from south Florida and the eastern Gulf of Mexico from the Florida Keys to Destin, Florida. García (2008a) also reported this species from Bermuda but the specimen may not be conspecific. *Anna florida* appears to be rarely encountered although one lot from Bonefish Key (FMNH 191310) contained 99 specimens.

Habitat: Dead specimens have been recorded from 28–100 m; live specimens have been collected from 2–50 m. Substrate unknown.

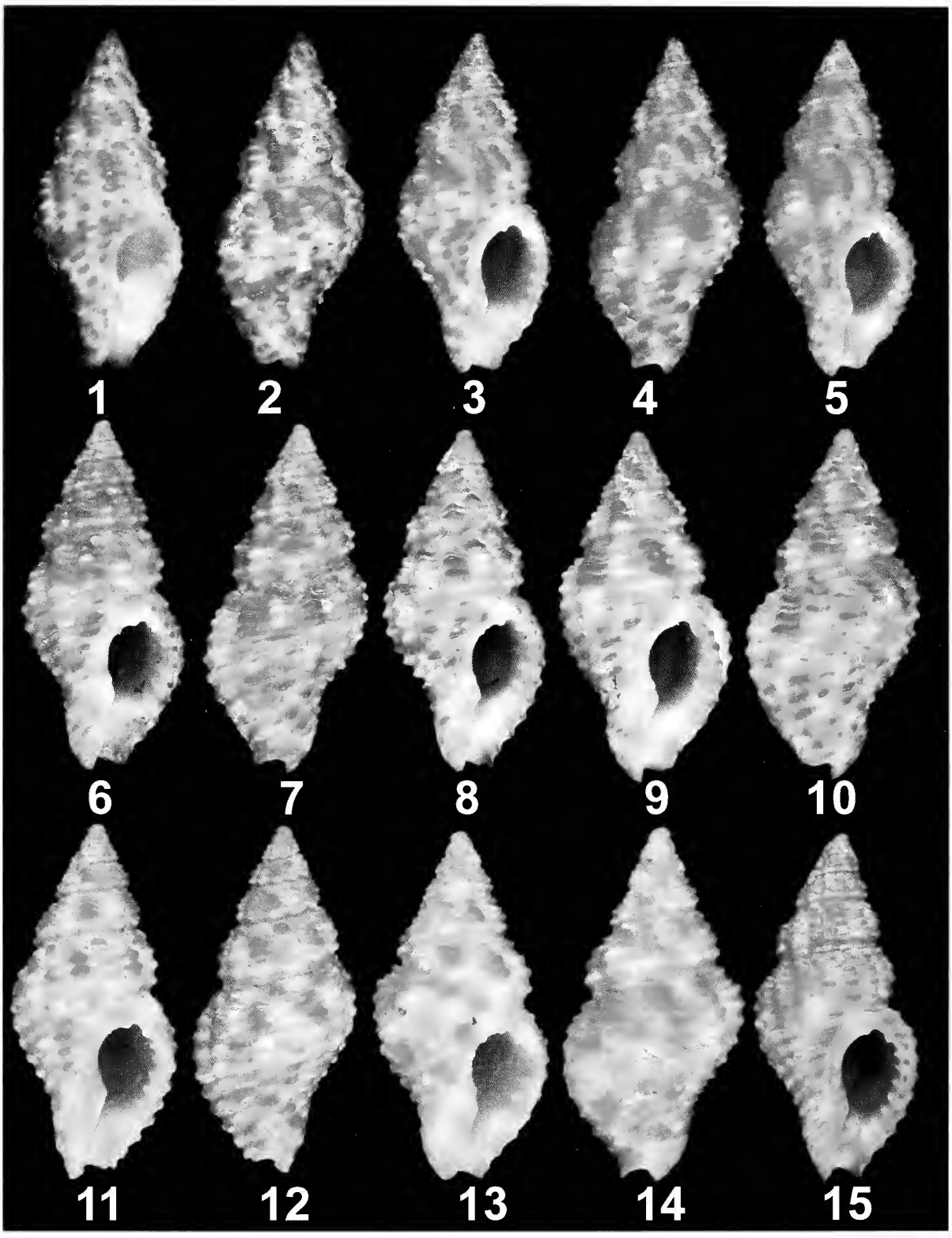
Etymology: Latin feminine noun *florida*, full of flowers, "in reference to the profusion of bright nodes that cover the surface of the shell. The epithet is also meant to evoke the State of Florida, whose name has the same provenance and where the new species seems to be most common" (García, 2008a).

Discussion: *Anna florida* is most similar to *A. willemsae*. They do not appear to be sympatric. *Anna florida* is larger and more fusiform than *A. willemsae*, often has more axial ribs on the penultimate whorl (7–10 in *A. willemsae* vs. 9–13 in *A. florida*), and a different color pattern consisting of orangish polka-dots in contrast to the brown blotches of *A. willemsae*.

Anna milleri (Usticke, 1959)
(Figures 16–24)

Bailya milleri Usticke, 1959: 67–68, pl. 2, fig. 21; Kaicher, 1985: No. 4385; Boyko and Cordeiro, 2001: fig. 4.
"*Bailya*" *milleri* Usticke, 1959.—Watters, 2007: 10.

Description: Average size 9.1 mm in length (min, 8.3; max, 10.0). Fusiform; spire ca. 50–60% total length. Protoconch small, of 1.5 smooth, uncolored whorls. Teleoconch of 5 whorls, weakly demarcated from protoconch. Teleoconch sculpture of ca. 15–16 rounded, narrow, widely separated, spiral threads, including siphonal canal, often with a single, minute, intercalated 2° thread. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely spaced, high ribs; ca. 9 ribs on penultimate whorl, ca. 8 ribs on last whorl, not including varix. Intersections of axial and spiral sculptured without elongated nodules. Terminal varix well-developed, somewhat constricted, wide. Aperture oval, outer lip



Figures 1–15. *Anna florida* García, 2008. **1–2.** Holotype, ANSP 418032, 14.2 mm, photo courtesy E. F. García. **3–4.** UF 150206, from the type locality, 14.3 mm. **5.** UF 150206, from the type locality, 14.4 mm. **6–10.** UF 70453, Bonefish Key, Monroe Co., Florida. **6–7.** 12.0 mm. **8.** 11.0 mm. **9–10.** 10.7 mm. **11–12.** UF 186142, 100 m, 200° off Sand Key, *Triton* Sta. 956, Monroe Co., Florida, 11.3 mm. **13–14.** UF 266955, off Miami, 80 m, Miami-Dade Co., Florida, 11.1 mm. **15.** UF 154765, off Destin, 28 m, Okaloosa Co., Florida, 12.8 mm.

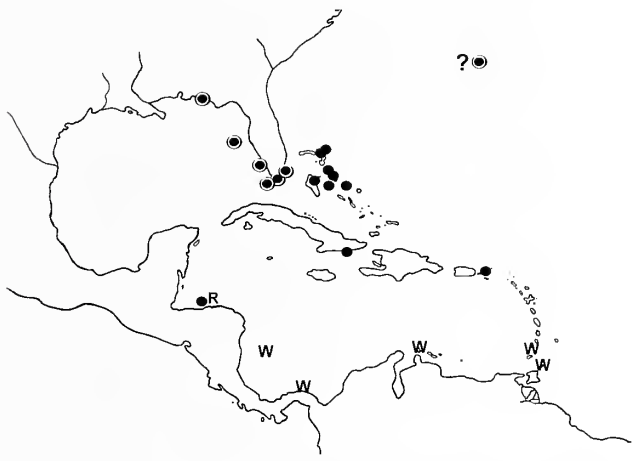


Figure 16. Distribution of *Anna florida* García, 2008 (bullseye), *Anna milleri* (Usticke, 1969) (solid), *Anna royalensis* new species (R), and *Anna willemsae* (De Jong and Coomans, 1988) (W).

with 7–8 lirate teeth. Columella angled at siphonal canal and bearing ca. 6 minute but distinct denticles along its length; parietal lip erect for most of its length. Siphonal canal short, open. Color white flushed with tan on axial ribs and with wide sub-peripheral white band. Primary spiral cords darker brown. Occasional specimens are uniformly white but still possess dark primary cords. Aperture white. Operculum oval, pale yellow, with anterior terminal nucleus. Redfern (2006: fig. 399c) illustrated a live animal; it is white with brown streaks and maculations. Radula and anatomy unknown.

Holotype: AMNH 193772, specimen not available for study, but figured in Kaicher (1985) and Boyko and Cordeiro (2001), the latter reproduced here.

Type Locality: Outer reef of Christiansted Harbor, St. Croix, US Virgin Islands.

Other Material Examined: Bahamas. HGL, Cat Island; HGL, 28 m, Long Cay, Exuma Islands; HGL, drift, Governor's Harbour, Eleuthera; HGL, drift, North Current Cut, Current Island, Eleuthera; HGL, 0.3–1 m, Joe's Creek, Abaco; CR 3597, 10445, both 0.5 m, Joe's Creek, Abaco, 26°37′ N, 77°16′ W; CR 3737, 9 m, Chub Rocks, Abaco, 26°44′ N, 77°13′ W. Cuba. UF 316419, Jauco, Santiago de Cuba Province. Honduras. EFG 17461, 10–13 m, Helene, E Roatán Island.

Distribution: This is a rare species. It has been found off the Bahamas, Cuba, the Virgin Islands, and Honduras. The holotype was found in shallow water but was apparently not live-taken. Usticke (1959: 68) remarked that "there were more of them, but just at that moment a terrific hailstorm(?) broke, so roiled the water that the others got away."

Habitat: Dead shells have been found to 28 m but live shells have been collected from beach drift to 9 m under rocks. The holotype was found on a reef.

Etymology: Named after Joe Miller, friend of Usticke.

Discussion: Usticke's original figure seems to be a drawing or retouched photograph and was poorly executed. Fortunately the type was re-illustrated by Kaicher (1985) and Boyko and Cordeiro (2001). Faber (2007) placed *Bailya marijkae* De Jong and Coomans, 1988, *Engina willemsae* De Jong and Coomans, 1988, and *Engina goncalvesi* Coltro, 2005, in synonymy of *A. milleri*, but I consider all to be valid species. As mentioned by Faber (2007) *Ricinula eximia* Reeve, 1846, supposedly from the Indo-Pacific Ocean, is extremely close to *A. milleri*. A syntype of *R. eximia* was illustrated in Cernohorsky (1978, fig. 54). Kaicher (1990, No. 5839) illustrated a different syntype of *R. eximia*, but it is not conspecific with the Cernohorsky specimen. Both syntypes of *R. eximia* have a more elongate shell that lacks the numerous denticles along the length of the columella found in *A. milleri*. I do not believe they are the same species. See Table 2 for a comparison with other *Anna*.

Anna milleri is most similar to *A. willemsae* but consistently differs in the following ways. The primary spiral cords of *A. willemsae* may be colored white or brown in the interaxial spaces but are always white as the pass over the axial ribs; in *A. milleri* the cords are dark regardless of their position. The spiral cords, both 1° and 2°, are better developed in *A. willemsae* than in *A. milleri*. *Anna milleri* is more coarsely sculptured and has a different color pattern than *A. royalensis* new species.

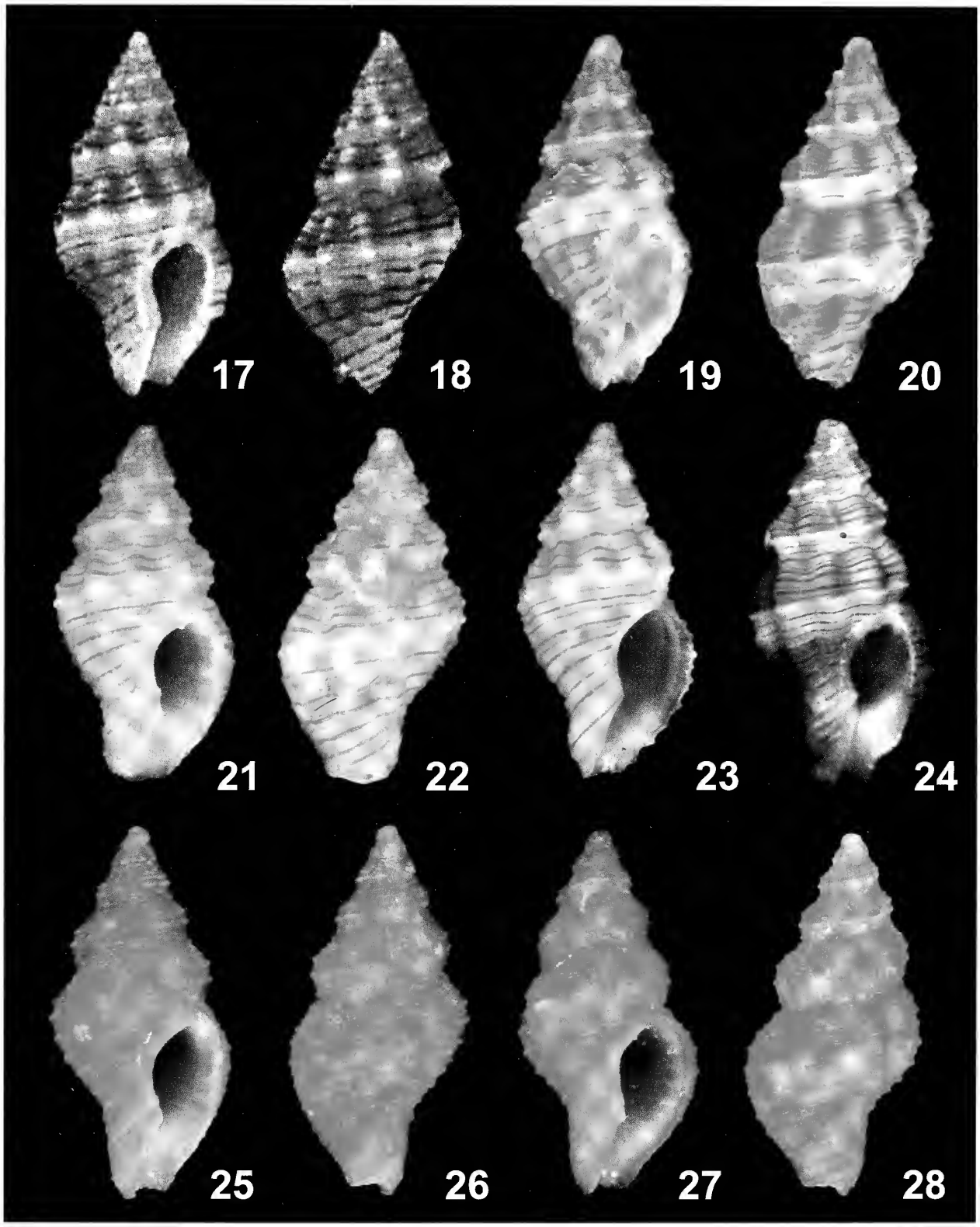
Anna royalensis new species
(Figures 16, 25–28)

Description: Shell 9.7–10.4 mm in length (holotype 10.4 mm in length). Fusiform; spire ca. 50–60% total length. Protoconch small, of 1.5 smooth, uncolored whorls. Teleoconch of 4.75 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 16 rounded, prominent, widely-separated, spiral threads, including siphonal canal, with intercalated 2° and 3° threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely-spaced, rounded, high ribs; 8 ribs on penultimate whorl, 7–8 ribs on last whorl, not including varix. Intersections of axial and spiral sculptured with elongated nodules formed from cords, most pronounced on periphery. Terminal varix well-developed, somewhat constricted, wide. Aperture oval, outer lip with 8 lirate teeth. Columella angled at siphonal canal and bearing 3–5 minute denticles along its length, becoming progressively stronger anteriorly; parietal lip erect for most of its length. Siphonal canal short, open. The color is tan, with darker axial ribs, and a vague, wide sub-peripheral pale band. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: UF 425837 (*ex* GTW).

Type Locality: Sand and coral rubble, 6.7 m, off Old Port Royal Harbour, SE Roatán Island, Honduras.

Paratype: BMSM 17977, 1 shell, 9.7 mm, from the type locality (*ex* GTW).



Figures 17–28. *Anna* species. **17–24.** *Anna milleri* (Usticke, 1959). **17–18.** Holotype, *Bailya milleri* Usticke, 1959, AMNH 193772, 10 mm, reproduced from Boyko and Cordeiro (2001). **19–20.** HGL, drift, Governor's Harbour, Eleuthera, Bahamas, 9.4 mm. **21–22.** HGL, drift, North Current Cut, Current Island, Eleuthera, Bahamas, 8.5mm. **23.** HGL, Cat Island, Bahamas, 8.4 mm. **24.** EFG 17461, 10–13 m, Helene, E Roatán Island, Honduras, 11 mm, photo courtesy E.F. García. **25–28.** *Anna royalensis* new species. **25–26.** Holotype, UF 425837, 10.5 mm. **27–28.** Paratype, BMSM 17977, 9.7 mm, from type locality.

Table 2. Shell characteristics of *Anna* species.

	Average length (max) mm	# axial ribs on penultimate whorl	# denticles on columella	# lirae on inner surface of outer lip	color
florida	14.2 (16.2)	9–13	3–5	7–9	White with orange-brown flammulations and dots and white band
milleri	9.0 (10.0)	9	6	7	Tan axials and white band, spiral cords brown
royalensis	10.4	8	5	8	Tan with darker axials and pale band
willemsae	10.2 (10.9)	7–10	5–6	6–7	Tan axials and white band, spiral cords brown, white over axials

Other Material Examined: Honduras. GTW 14020a, 4-5 m, Utila Island (three shells).

Distribution: Known only from the type locality and Utila Island. The holotype and paratype are from 6.7 m and are freshly dead shells.

Habitat: Freshly dead shells have been found in sand under coral rubble at 5-7 m. The living Utila specimens were found at 4-5 m on the underside of a partially buried, dead coral slab in silty sand (B. Bessc, pers. comm., 2009).

Etymology: After the type locality, Old Port Royal.

Discussion: This appears to be a Bay Islands endemic. It differs from the related *Anna willemsae* in having well-formed 2° and 3° spiral cords, in the weaker columellar denticles, its more biconical shape, and its nearly monochromatic color pattern. *Anna milleri* also occurs at Roatán Island (Figure 24) but is easily separable by its color pattern and less coarse sculpture; it may occur in deeper water there than *A. royalensis*. *Anna royalensis* differs from *A. florida* in being generally smaller, less nodulose, and in having a different color pattern. See Table 2 for a comparison.

Anna willemsae (De Jong and Coomans, 1988)
(Figures 16, 29–40)

Engina willemsae De Jong and Coomans, 1988: 83, pl. 38, fig. 452; Faber, 2007: 74, figs. 9,10 [holotype, in synonymy of *Bailya milleri* Usticke, 1959].
?*Pollia* sp. Redfern, 2001: 94, pl. 43, figs. 399a, b, c.
Engina milleri (Usticke, 1959).—Faber, 2007: 74–75, figs. 13–16 [in synonymy].

Description: Average size 10.2 mm in length (min, 9.3; max, 10.9). Fusiform; spire ca. 50–60% total length. Protoconch small, of 1.5 smooth, uncolored whorls. Teleoconch of 5 whorls, weakly demarcated from protoconch. Teleoconch sculpture of ca. 13–15 rounded, prominent, widely separated, spiral threads, including siphonal canal, with intercalated 2° threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely spaced, high ribs; ca. 7–10 ribs on penultimate whorl, 6–8 ribs on last whorl, not including varix. Intersections of axial and spiral sculptured with strong, elongated nodules. Terminal varix well developed, somewhat constricted, wide. Aperture oval, outer lip with 6–7 lirate teeth. Columella angled at siphonal canal and bearing 5–6 minute but distinct denticles along its length; parietal lip erect for most of its length. Siphonal canal short, open. Color tan, orangish, or brown with wide sub-peripheral pale band. Primary spiral cords brown between axial ribs white as they pass over the axial ribs. Aperture white. Operculum leaf-shaped, tan, with anterior terminal nucleus. Radula and anatomy unknown.

Holotype: ZMA 3.87.085.

Type Locality: Aruba, harbour.

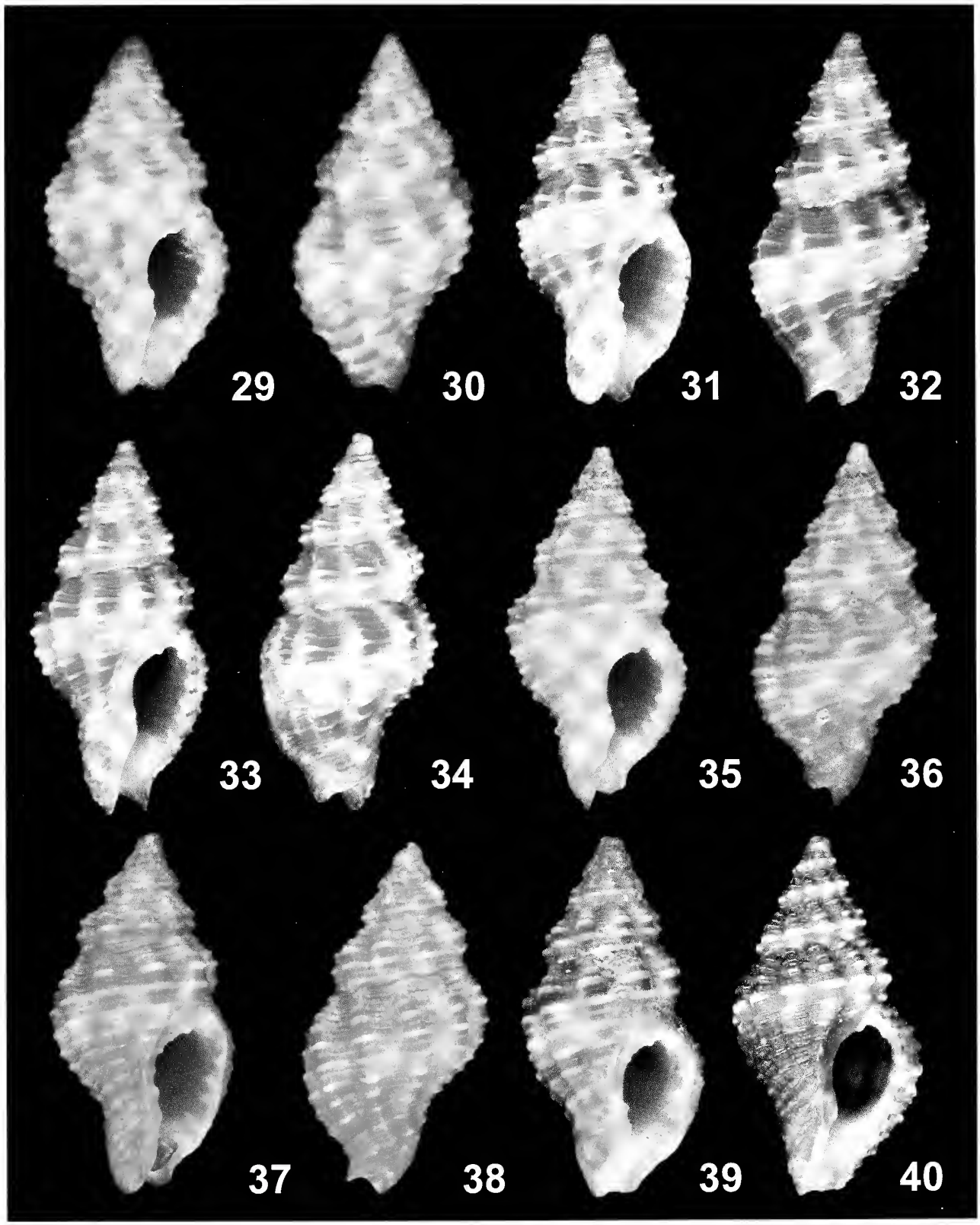
Other Material Examined: Panama. UF 397258, Devil's Beach; UF 425826, Isla Galeta; UF 160582, East Colon Island. Colombia. GTW 7371b, 40–60 m, off Cayos de San Andrés. Trinidad and Tobago. UF 425828, Scarborough, Tobago. St. Vincent and Grenadines. Phil Fallon coll., Clifton Harbour, Union Island. Netherlands Antilles. Frère Fredericus Verberne coll., Aruba. ?Venezuela. GTW 7371c, 12–17 m, Coché, Isla Margarita.

Distribution: This is a rare species in the southern Caribbean Sea. It has been found off Panama, Cayos de San Andres, Tobago, and Aruba. Most specimens seen are from Cayos de San Andres. Possibly the Bahamas and off Venezuela as well (see Discussion, below).

Habitat: Most specimens examined were worn. Freshly dead shells were recorded from 40–60 m. Nothing is known of the substrate.

Etymology: Named after Mrs. Ineke Peeters-Willems, collector of the type specimen.

Discussion: Some of the specimens referred to here as *A. willemsae*, such as the *Pollia* sp. of Redfern (2001, pl. 43, figs. 399a, b, c) from the Bahamas, and specimens from Isla Margarita, Venezuela (Figure 40), will probably warrant description as a new species when more examples are found. They differ in having much darker coloration and coarser sculpture than either *A. milleri*



Figures 29–40. *Anna williamsae* (De Jong and Coomans, 1988). **29–30.** Holotype, ZMA 3.87.085, 10.4 mm. **31–34.** GTW 7371b, 40–60 m, off Cayos de San Andrés, Colombia. **31–32.** 9.4 mm. **33–34.** 9.2 mm. **35–36.** UF 425828, Punta Galeta, Isla Galeta, Panama. 10.7 mm. **37–39.** UF 397258, Devils Beach, Panama. **37–38.** 9.9 mm. **39.** 11.0 mm. **40.** GTW 7371c, 12–16 m, Coché, Isla Margarita, Venezuela, 9.2 mm.

or *A. willemsae*. For now this form has only been recorded from the extreme north and south of the Caribbean Sea.

See under *A. milleri* for a comparison with that species. See Table 2 for a comparison with other western Atlantic *Anna*.

Genus *Antillophos* Woodring, 1928

Tritiaria (*Antillophos*) Woodring, 1928: 2, 6, 259.

Type Species: *Cancellaria candeana* d'Orbigny, 1842, by original designation.

Description: Small to medium-sized (to 40 mm). Fusiform; aperture 50–70% of shell length. Protoconch of 1.5 small, smooth, conical whorls with sharp peripheral keel. Teleoconch sculpture of spiral threads and axial ribs. Previous varices may be present. Terminal varix thickened and often wide. Aperture lirate within outer lip. Columella with denticles bounding anal and siphonal canals; some species with additional denticles along length of columella. Columella distinctly angled at siphonal canal. See Table 1 for comparison with other genera.

Discussion: The genus *Phos* has been divided into several subgenera, some (including *Antillophos*) now regarded as full genera. The distinctions are based on minor differences in protoconch morphology, teleoconch sculpture, and the presence or absence of lirae on the columella. This combination of characteristics does not seem to lead to a natural grouping. Even the protoconch differences are minor, based as they are on the number of keels or spiral threads (one in *Antillophos*, up to four in *Metaphos*, etc.). But the Senegalese "*Phos*" *grateloupinaum* Petit, 1853, has a single spiral thread for one whorl but between two and five keels are added subsequently. Adding to the confusion, numerous Philippine species have recently been assigned to *Antillophos* (Fraussen and Poppe, 2005), some having a protoconch with two spiral keels whereas others have only one. At this time it is difficult to differentiate one *Phos*-like supraspecific taxon from another. Dall (1889: 178) commented on the western Atlantic *Phos*: "But a very small amount of investigation in this case, as in many others, will show that, apart from the bare shells, there is much yet to be learned about almost all of these animals."

Tritiaria is considered a fossil genus and the possible precursor to *Antillophos* (Haasl, 2000). Numerous fossil species have been assigned to *Antillophos* but future work is needed to separate them into *Tritiaria* and *Antillophos*.

Phos elegans Guppy, 1866, is a name occasionally applied to several of the *Antillophos* described here. It resembles *A. candeanus* more than any other species. However, it is a Miocene species [and not a *nomen dubium* as previously stated (Watters, 2008)].

Antillophos bahamasensis Petuch, 2002
(Figures 41–42, 56)

Antillophos bahamasensis Petuch, 2002: 63–64, figs. 2a, b; Watters, 2008: 5, fig. 1.

Description: Shell 18–20 mm in length (holotype 18 mm in length). Fusiform; spire ca. 60% of total length. Protoconch worn, conical, of ca. 2.25 smooth whorls with evidence of keel at periphery. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with narrow, widely spaced, flat, spiral cords separated by wide intervals, ca. 17 on last whorl. Interspaces with single, fine, 2° spiral thread. Axial sculpture of widely spaced, low, rounded ribs, ca. 19 on last whorl (excluding varix) and ca. 17 on penultimate whorl. Varices well-developed, about one varix every 1/3–1/4 whorl except for last whorl. Terminal varix low, wide, crossed by numerous axial ribs. Intersections of axial and spiral sculpture form pustulose, rachet-like sculpture. Aperture elongate-oval, with one plication anteriorly; anal canal set off by two denticles. Outer lip with ca. 16 lirae deep within mouth, with intercalated 2° ones. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" small and shallow. Holotype somewhat bleached and worn, colored white; paratype in the Petuch collection apparently retains some color as Petuch (2002: 63) stated "color pale tan with 3 darker tan bands and with spire whorls being darker tan." Aperture white. Radula, operculum, and anatomy unknown.

Holotype: UF 277198.

Type Locality: Off Victory Cay, Bimini Chain, Bahamas.

Paratype: Petuch coll., from type locality.

Distribution: Known only from the type locality.

Habitat: Both specimens appear to be dead shells. Although the type locality did not include a bathymetric range, in the discussion of the species is included the statement "depths of 35 m." Substrate unknown.

Etymology: From the Bahamas.

Discussion: The type is a slightly worn, bleached specimen. It is similar to *A. chazaliei*, but more elongate and with finer sculpture. The collection of additional material may eventually necessitate the synonymizing of *A. bahamasensis* with *A. chazaliei*. See Table 3 for a comparison.

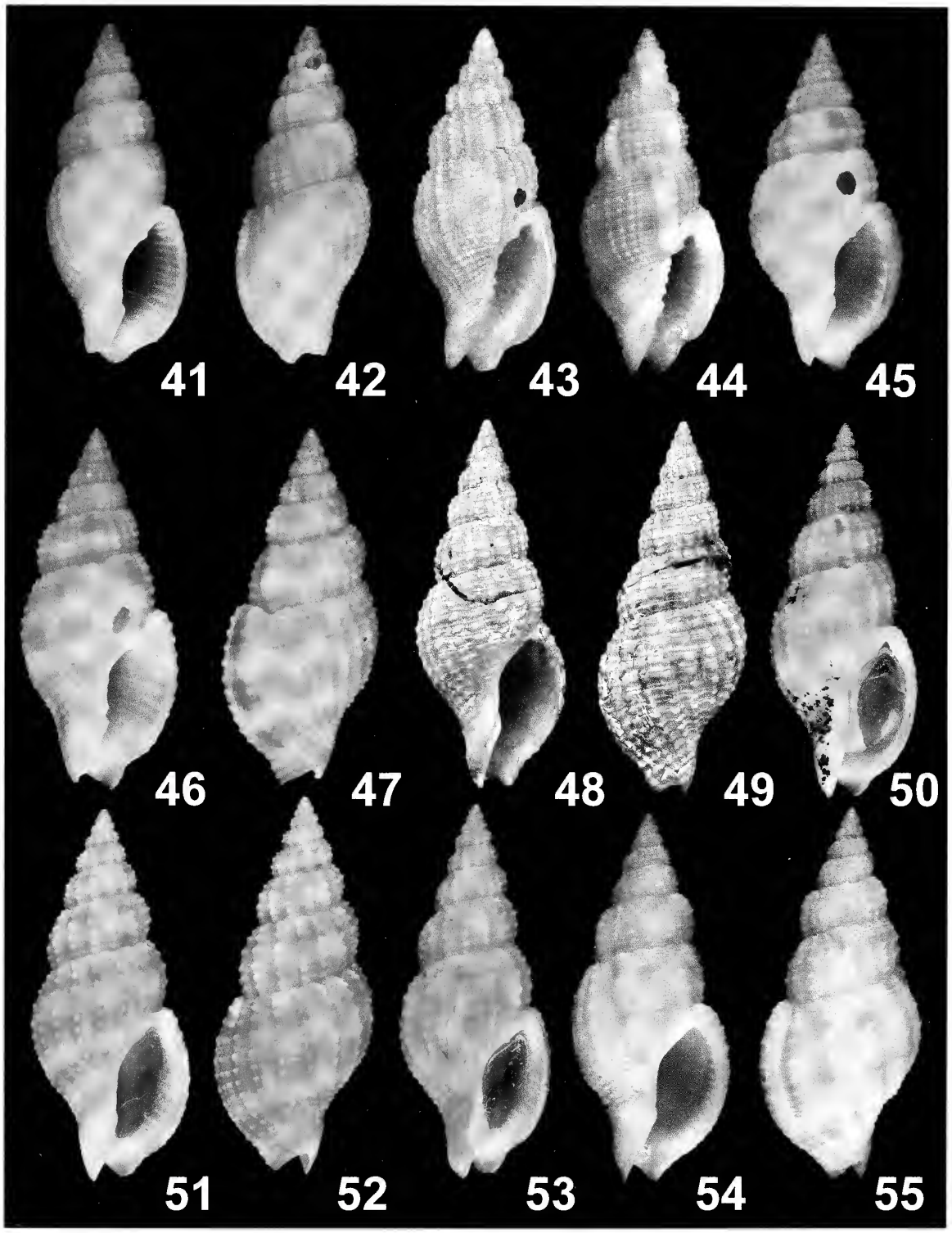
Antillophos beauii (Fischer and Bernardi, 1857)
(Figures 57–69)

Phos beauii Fischer and Bernardi, 1857: 358, pl. 12, figs. 8, 9; Tryon, 1881: 219, pl. 84, fig. 533; Kaicher, 1985: No. 4318.

Phos beaui [*sic*] Fischer and Bernardi, 1857.—Dall, 1889a: 15, 178–179.

Antillophos beaui [*sic*] (Fischer and Bernardi, 1857).—McGinty and McGinty, 1957: 40; Abbott, 1974: 220; Abbott and Dance, 1982: 167; Watters, 2008: 5, fig. 3.

Description: Average 29.1 mm in length (min, 25.1; max, 31.8). Fusiform; spire ca. 60% of total length. Protoconch minute, brown or purple, conical, of ca. 2.25 smooth whorls with sharp keel at periphery; first whorl



Figures 41–55. *Antillophos* species. **41–42.** *Antillophos bahamasensis* Petuch, 2002. Holotype, UF277198, 18.0 mm. **43–47.** *Antillophos oxyglyptus* (Dall and Simpson, 1901). **43.** Holotype, USNM 159696, 17 mm, photos courtesy of Y. Villacampa (USNM). **44.** Holotype of *Antillophos bayeri* Petuch, 1987, USNM 859854, 17 mm, photos courtesy of Y. Villacampa (USNM). **45.** GTW 9216a, 167–200 m, W Sandy Lane Bay, Barbados, 20.0 mm **46–47.** HGL, 167–200 m, W Sandy Lane Bay, Barbados, 24.3 mm. **48–55.** *Antillophos smithi* (Watson, 1885). **48–49.** Holotype, BM(NH) 1887.2.9.751, 34 mm, photos courtesy of A. MacLellan (BM(NH)). **50.** HGL, 200–233 m, W Barbados, 36.5 mm. **51–52.** GTW 9163b, 230–260 m, off Roatán Island, Honduras, 30.4 mm. **53.** HGL, 230–260 m, off Roatán Island, Honduras, 31.2 mm. **54–55.** *Antillophos freemani* Petuch, 2002. Holotype, UF 277099, 19 mm.

Figure 56. Distribution of *Antillophos bahamasensis* Petuch, 2002 (bullseye) and *Antillophos chazaliei* (Dautzenberg, 1900) (solid).

partially sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. First four teleoconch whorls sculptured with spiral incised grooves; grooves lost on subsequent whorls except for 7–10 grooves on siphonal canal. Axial sculpture of widely spaced low ribs with occasional varices; 9–14 low ribs on last whorl excluding varix. Varices acutely shouldered, may occasionally line up with previous whorls or may be at random. Terminal varix narrow, set back a short distance from outer lip. Last 1–3 whorls nearly smooth, polished. Aperture elongate-oval, with two plications at the siphonal canal; anal canal set off by two denticles. Outer lip with 15–25 fine lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" wide and shallow. Colored with broad bands of different shades of tan separated by narrow white bands; bands darkest on varices. Aperture white. Operculum leaf-shaped, yellow or tan, with anterior terminal nucleus. Dall (1889: 178–179) described the animal in detail: "The soft parts are white, dotted with blackish toward the middle line of the foot above, and with the end of the siphon very dark brown. The eyes are very large in proportion to the size of the animal, are mounted on large long stout peduncles, from the inner side of the distal end of which proceed very slender acute tentacles. The foot is large, thin, with an entire edge and pointed linuiform tail-end." Radula unknown.

Type(s): The specimen illustrated in the original description represents the species universally recognized as *Phos beauii*. Dance (1966) stated that Fischer's types were housed at BM(NH) and MNNH. No type was found at BM(NH) (*fide* A. MacLellan, pers. comm., 2008). MNHN has a single specimen labeled as a syntype. That specimen is not the same as that depicted in the figure and in fact is an example of the species later called *Phos oxyglyptus* Dall and Simpson, 1901. The original description of *Phos beauii* does not mention multiple specimens and it is uncertain how the MNHN specimen became known as a syntype. If another specimen existed, presumeably the illustrated example, it appears to be lost. If we identify *Phos beauii* with the remaining specimen at MHNH then *Phos beauii* becomes a senior synonym of *Phos oxyglyptus*. In addition such action would leave the species now known as *A. beauii* without a valid name. In the interest of taxonomic stability **I designate the original figures (Fischer and Bernardi, 1857: pl. 12, figs. 8, 9; reproduced here, figs. 57, 58) as the lectotype of *Phos beauii*** (see ICZN Recommendations 73F and 74B).

Type Locality: Marie-Galante [E of Guadeloupe]. Collected in fishing traps.

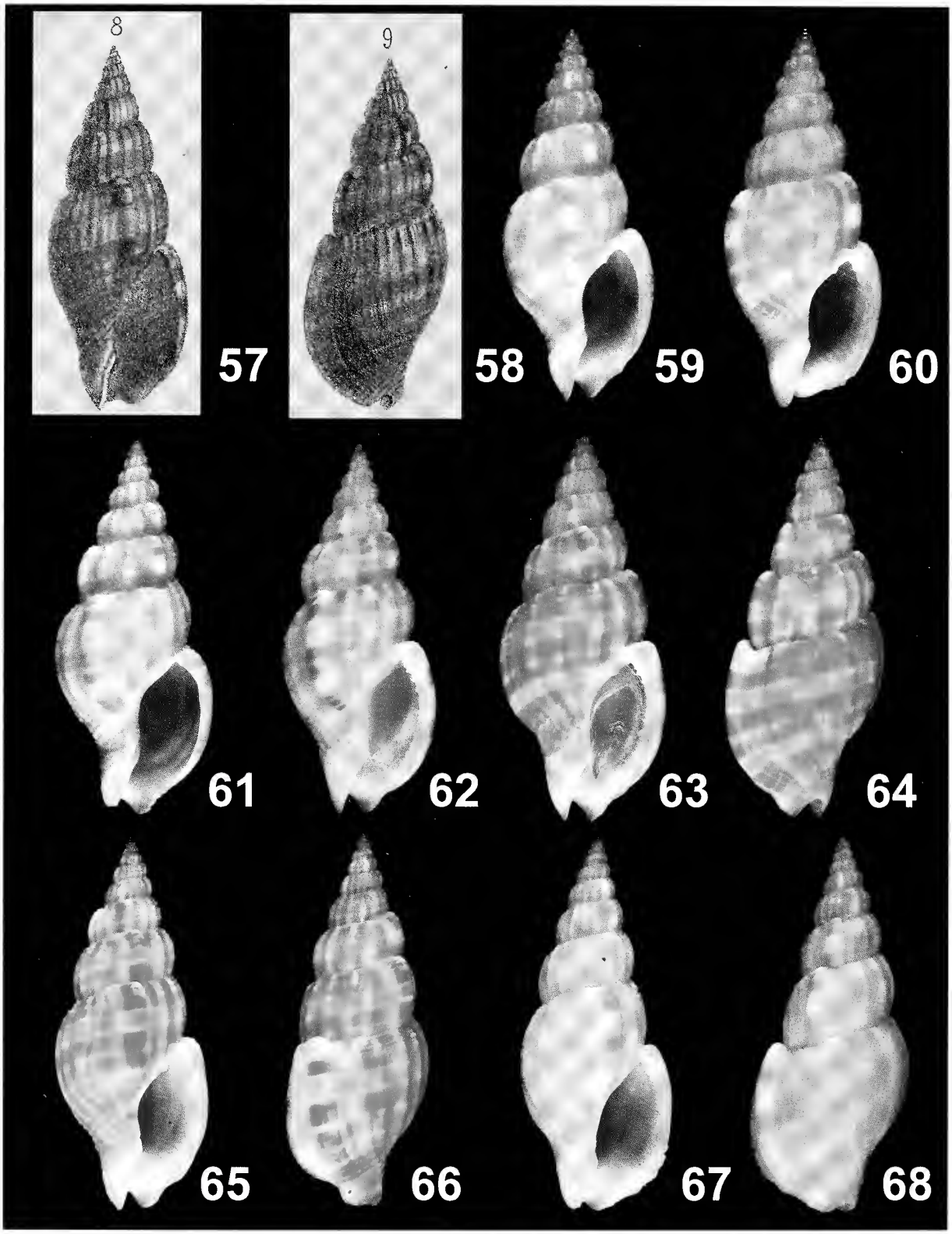
Other Material Examined: Florida. UF 154766, *Triton* Sta. 83, 30 m, off Palm Beach Pier, Palm Beach Co. Bahamas. EFG 5358, 300 m, 26°49′ N, 77°01′ W. Dominican Republic. GTW 5891d, 200 m, off La Romana. Puerto Rico. HGL, fishtrap, 40–50 m, off Cabo Rojo. Guadeloupe. UF 121284, 160 m, off Port Louis; EFG 7999, traps, 150 m. Barbados. GTW 5891a, 167 m, off St. James; GTW 5891b, 180 m, S shore; GTW 5891c, 180 m, W coast. Colombia. UF 212353, 67–83 m, Guajira Province.

Distribution: Off SE Florida, the Bahamas, the Dominican Republic, Puerto Rico, Guadeloupe, Barbados, and Venezuela. Most specimens in collections are from Barbados.

Habitat: Dead shells are sporadically recorded from 30 m (rare) to 200 m and live specimens have been

Table 3. Shell characteristics of *Antillophos* species.

	Average length (max) mm	# teleoconch whorls	# axial ribs on penultimate whorl	Predominant sculpture on final whorl	# lirations in outer lip
bahamasensis	18	6.5	17	pustulose	16
beauii	29.1 (31.8)	6.5	9–14	nearly smooth	15–25
candeanus	24.1 (31.8)	6.5	12–14	serrate	8–11
chazaliei	12.7 (24.0)	5.5	8–12	serrate	12–14
oxyglyptus	20.4 (24)	6.5	16–18	pustulose	11–12
smithi	28.3 (36.6)	6.5	12–18	pustulose	16–21
verriculum	31.0 (34.0)	6.5	9–17	serrate	12–15
virginiae	23.6 (32.2)	6.5	13–20	pustulose	10–17



Figures 57–68. *Antillophos beauii* (Fischer and Bernardi, 1857). **57–58.** Lectotype, Fischer and Bernardi, 1857: pl. 12, figs. 8, 9. **59.** UF 121284, 160 m, off Port Louis, Guadeloupe, 26.1 mm. **60.** UF 212353, 67–83 m, Guajira Province, Colombia, 21.5 mm. **61.** UF 154766, 30 m, off Palm Beach Pier, Palm Beach Co., Florida, 22.9 mm. **62.** GTW 5891d, 200 m, off La Romana, Dominican Republic, 28.0 mm. **63–64.** GTW 5891c, 167 m, W Barbados, 30.7 mm. **65–66.** GTW5891b, 180 m, S Barbados, 29.7 mm. **67–68.** HGL, 160–200 m, off Cabo Rojo, Puerto Rico, 31.9.

Figure 69. Distribution of *Antillophos beauii* (Fischer and Bernardi, 1857).

taken in 167–200 m. McGinty and McGinty (1957) reported this species off Palm Beach in 50–60 fathoms on rubble patches and mud. Several specimens (including the holotype) have been caught in baited fishing traps suggesting that the species may be a scavenger.

Etymology: Named after Commander Beau, French "chef de bataillon d'infanterie," collector in Guadeloupe. Although several species are named after Beau, it does not appear that we know much about him.

Discussion: This beautiful species is the most easily recognized *Antillophos* in the western Atlantic. Its large size, dark protoconch, lack of spiral sculpture on later whorls, and polished appearance immediately set it apart from all others. *Antillophos smithi* is of similar shape and size but is densely sculptured with minute pustules. See Table 3 for a comparison with other species.

Antillophos candeanus (d'Orbigny, 1842)
(Figures 70–85)

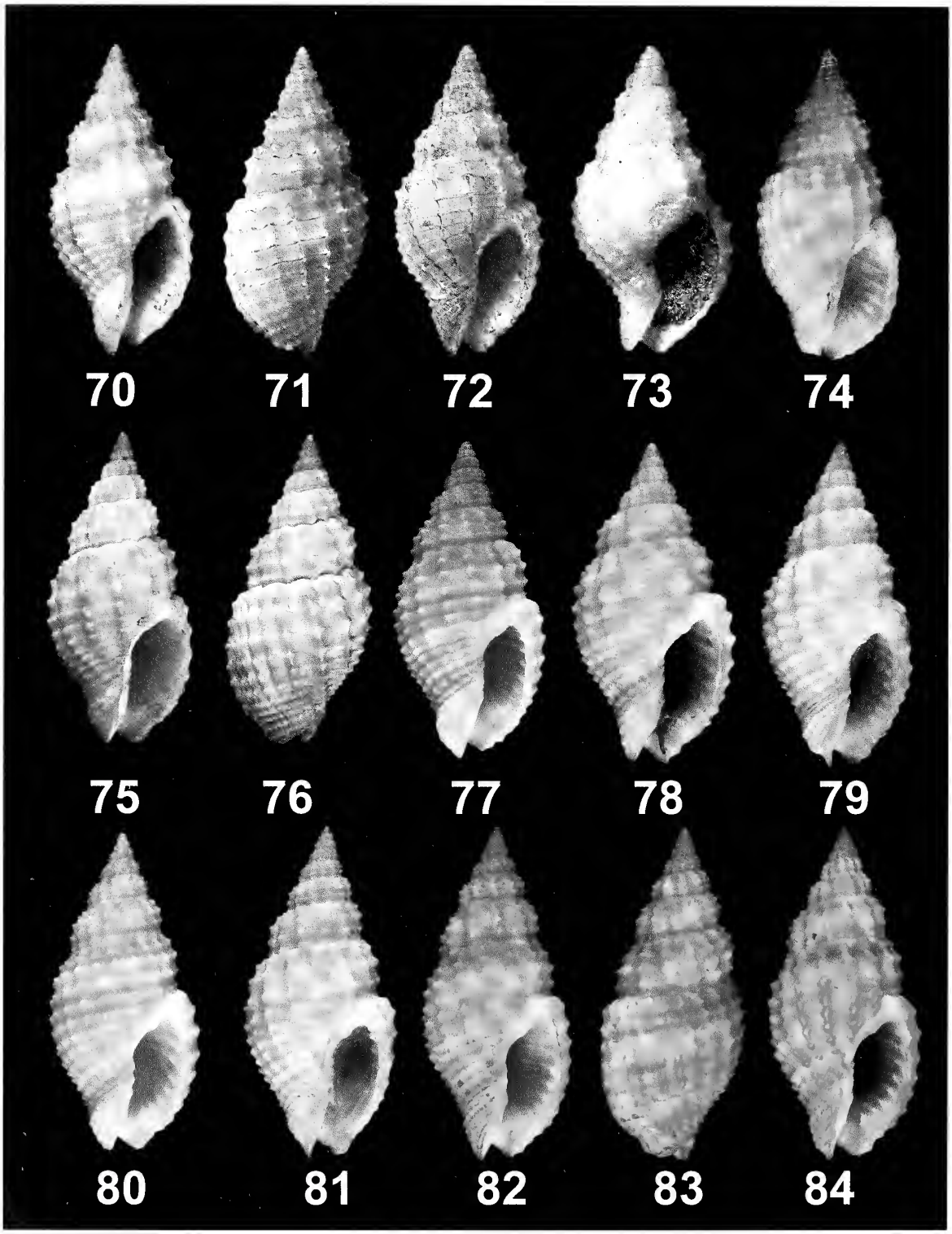
Cancellaria candeana d'Orbigny, 1842: pl. 23, figs. 4–6.
Cancellaria candei d'Orbigny, 1847: 129 [unjustified emendation].
Phos antillarum Petit, 1853: 238, 242–243, pl. 8, fig. 9; Tryon, 1881: 219, pl. 84, fig. 531 [in synonymy of *Phos veraguensis* Hinds, 1843]; Dall, 1889a: 179 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842]; Dautzenberg, 1900: 180; Maury, 1922: 58 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842]; Abbott, 1974: 220 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842]; Rios, 1985: 102 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842]. Rios, 1994: 120 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842].
Phos candei (d'Orbigny, 1842).—Arango, 1878: 201; Tryon, 1881: 219 [in synonymy of *Phos veraguensis* Hinds, 1843]; Dall, 1889a: 15, 179 [in part]; Maury, 1922: 58.
? *Phos candei* (d'Orbigny, 1842).—Dall, 1889b: 116–117; Dall and Simpson, 1901: 401; Henderson, 1914: 120.
Antillophos candei (d'Orbigny, 1842).—Abbott, 1954: 231–232 [in part], pl. 25u; Warmke and Abbott, 1961: 115, pl. 21, fig. h; Abbott, 1974: 220 [in part]; Humphrey, 1975: pl. 17, fig. 13; Sarasua and Espinosa, 1984: 8, fig. 4d.
Antillophos cf. adelus (Schwengel, 1942).—Petuch, 1987: pl. 24, figs. 7, 8; Merlano and Hegedus, 1994: 188, fig. 716.
Antillophos candeanus (d'Orbigny, 1842).—Robin, 2008: 182, fig. 7; Watters, 2008: 5, figs. 4, 5.

Description: Average 24.1 mm in length (min, 16.9; max, 31.8). Fusiform; spire ca. 50% of total length. Protoconch minute, white, conical, of ca. 2.25 smooth whorls with sharp keel at periphery; first whorl sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with weak, narrow, widely spaced spiral cords separated by incised grooves; ca. 12 cords on last whorl. Axial sculpture of widely spaced, low, rounded ribs separated by concave spaces; ca. 12 ribs on last whorl, excluding varix, and 12–14 ribs on penultimate whorl. Previous varices absent or not differentiated from axial sculpture. Terminal varix low, not well-differentiated, very wide, crossed by numerous axial ribs. Intersections of axial and spiral sculpture form rachet-like, posterior pointing serrations. Aperture elongate-oval, with 2–3 weak plications at siphonal canal, anal canal set off by two weak denticles. Outer lip with 8–11 lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" small but deep. Colored white or off-white with three vague tan or pinkish bands below suture, below periphery, and on siphonal canal, bands darkest on varices, or all serrations tinged with tan, or with dark subsutural band. Aperture white or faintly purple. Operculum rhomboid, yellow or tan, with anterior terminal nucleus. Dall (1889: 179) described the animal: "The soft parts and operculum are exactly like those of *Phos beaui*, but there is less of the blackish dotting, even the siphon has not much." Radula unknown.

Types: *Cancellaria candeana* d'Orbigny, 1842, syntypes BM(NH) 1854.10.4.349, 3 shells, 13, 19, 22 mm length; *Phos antillarum* Petit, 1853, syntype MNHN, unnumbered, 1 shell, 28.5 mm length (listed as holotype by Fischer-Piette, 1950: 15).

Type Locality: (*candeanus*) Martinique; (*antillarum*) La Guayra (Amérique meridionale) [La Guajira, Venezuela].

Other Material Examined: Florida. UF 239638, Palm Beach, Palm Beach Co.; UF 150998, 5 m, off Treasure Island, North Inlet, Palm Beach, Palm Beach Co.; UF 12724, 7.5 m, Lake Worth Inlet, Palm Beach Co.; BMSM 8098, Lake Worth, Palm Beach Co.; UF 12726, 40 m, off Delray Water Tank, Palm Beach Co.; BMSM 38499, Pompano Beach, Broward Co.; UF 157578, 20 m, Pompano Beach fill, Broward Co; EFG 19335, 20–27 m, off Dania, Broward Co.; HGL, dredged near Fowey Rocks, Miami-Dade Co.; FMNH 249643, 80 m, SW of Sombrero Light, Monroe Co.; GTW 12722i, 42 m, near Sombrero Light, Monroe Co; FMNH 170993, 100 m, off Dry Tortugas; UF 126253, 40 m, off Key West, Monroe Co.; EFG 8011, 400 m, off Florida Keys; UF 126252, 50 m, NE of Dry Tortugas, 25°00′ N; UF 170845, off SW Florida, 14 m, 25°00′ N,



Figures 70–84. *Autillophos caudcanus* (d'Orbigny, 1842). **70–73.** Syntypes of *Cancellaria caudcanus* d'Orbigny, 1842, BM(NH) 1854.10.4.349, photos courtesy of A. MacLellan (BM(NH)). **70–71.** 13 mm. **72.** 19 mm. **73.** 22 mm. **74.** HGL, 100 m, N coast of Tobago, 27.4 mm. **75–76.** *Phos autillarum* Petit, 1853, syntype MNHN, unnumbered, 28.5 mm. **77.** UF 281266, Scarborough, Tobago, 24.4 mm. **78.** UF 158171, 25 m, Grand Mal Bay, Grenada, 22.1 mm. **79.** UF 126253, 40 m, off Key West, Monroe Co., Florida, 27.5 mm. **80.** UF 239635, Puerta Plata, Dominican Republic, 30.1 mm. **81.** UF 171240, 60 m, off Naples, Collier Co., Florida, 28.4. **82–83.** GTW 4331a, 240 m, off Matanzas, Matanzas Province, Cuba, 31.8 mm. **84.** HGL, 20 m, off Cap Salomon, Martinique, 28.4 mm.

Figure 85. Distribution of *Antillophos candeanus* (d'Orbigny, 1842).

Monroe Co.?; BMSM 8099, Florida Straits; UF 261578, 52 m, Gulf of Mexieo, 25°40′–25°20′ N, Monroe Co.?; UF 260953, 50–60 m, Gulf of Mexico, 25°00′–26°00′ N, Monroe Co.?; UF 260194, 50–60 m, Gulf of Mexico, 25°40′ N, Monroe Co.?; UF 260836, 130–140 m, Gulf of Mexico, 25°31′ N, Monroe Co.?; UF 258981, 68 m, W coast of Monroe Co., 24°04′ N; UF 171240, 60 m, off Naples, 26°10′ N, Collier Co.; UF 260852, W of Sarasota, Sarasota Co.; OSUM 3490, 117 m, off St. Petersburg, Pinellas Co. Bahamas. UF 176628, 2–12 m, S Cat Cay, Bimini. Cuba. UF 266940, Guanuhaya, Sancti Spíritus Province; UF 266953, Varadero, Matanzas Province; UF 126256, 266952, both 60 m, off Matanzas Bay, Matanzas Province; GTW 4331a, 240 m, Matanzas, Matanzas Province. Dominiean Republie. UF 187500, 239635, 383455, all Puerto Plata; GTW 12722e, in fish nets, 42 m, off Las Salinas; UF 352849, 30–40 m, off Punta Oeoa; UF 171516, Santo Domingo. Puerto Rieo. HGL, harbor dredging, W eentral shore of San Juan Harbor; UF 164327, 100 m, Punta Jiguero; UF 154750, 163095, both Mayaguez Harbour; UF 163094, Mayaguez Dock; UF 126249, Ponce Bay. US Virgin Islands. UF 362721, Water Island. British Virgin Islands. GTW 12722g, GTW 12722h, both 1 m, West End, Tortola. Martinique. GTW 12722b, 25 m, near Grande Anse; HGL, 20 m, off Cap Salomon. Grenada. UF 158171, 25 m, Grand Mal Bay. Trinidad and Tobago. HGL, in fish pot, 100 m, off N coast, Tobago; UF 281266, 352850, both Scarborough, Tobago. Honduras. Phil Fallon coll. 10809233, 30–38 m, SE of Morat Island, off E end of Roatán Island BMSM 8102, "Caribbean Sea."

Distribution: Recorded from the southern half of Florida through the Greater and Lesser Antilles, one record eaeh from the Bahamas, Honduras, and Venezuela. Dall (1889b) listed "*Phos candei*" from Hatteras, North Carolina, but this seems to be a reference to *A. virginiae*, based on other records.

Habitat: Dead shells have been found in depths from 5 to 240 m, but most records are from 20–60 m. Live and freshly dead specimens have been taken in 8–40 m. It lives in somewhat shallower water than *A. virginiae*. It appears to be locally common; over 50 specimens have been taken in a single sample. Substrate unknown.

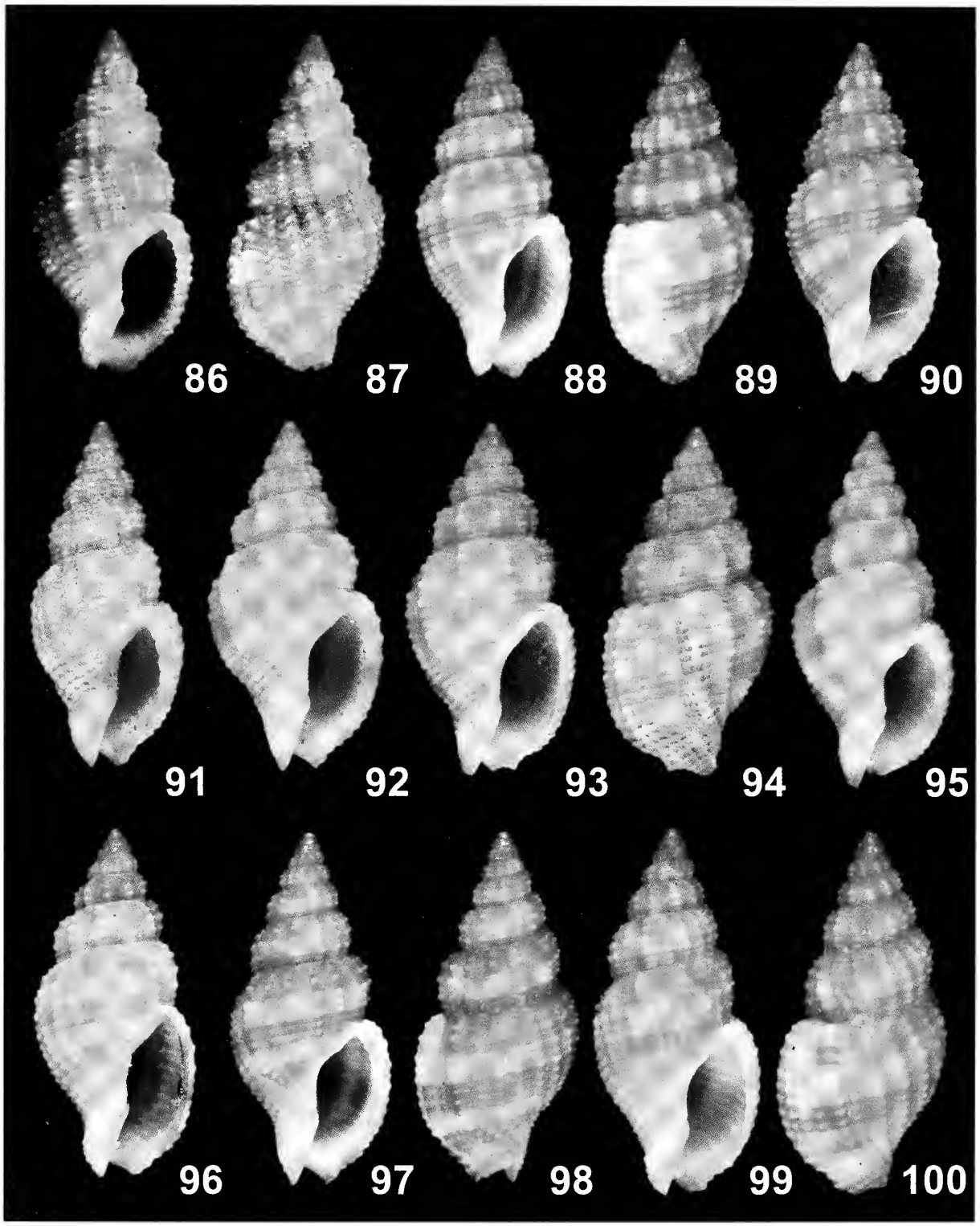
Etymology: Named after Ferdinand de Candé (1801–1867), Cuban naturalist and contemporary of d'Orbigny.

Discussion: The misused name "*candeanus/candei*" is the most commonly applied *nomen* for nearly any western Atlantic *Antillophos*. This species is easily differentiated from all others by its medium size, solid bullet-shape, coarse serrate sculpture, and fewer lirae inside the outer lip. The commonly confused *A. virginiae*, *A. oxyglyptus*, and *A. smithi* all have mueh finer sculpture that is more nodulose than serrate. *Antillophos chazalici* resembles a miniature version of *A. candeanus*, often being half the size or less of *A. candeanus*. Although *A. candeanus* does rarely occur in south Florida, the commonly dredged species there almost universally referred to as "*candeanus*" is aetually *A. virginiae*. See Table 3 for a comparison with other speeies.

Antillophos chazalici (Dautzenberg, 1900)
(Figures 56, 86–100)

Phos candei (d'Orbigny, 1842).—Tryon, 1881: pl. 84, fig. 534 [misidentification]; Dall, 1889a: 179 [in part].
Phos chazalici Dautzenberg, 1900: 181–182, pl. 9, fig. 7.
Bailya parva (Adams, 1850).—Merlano and Hegedus, 1994: 186, fig. 705 [misidentification].
Antillophos candei (d'Orbigny, 1842).—Abbott, 1974: 220 [in part], fig. 2425 [misidentification]; Merlano and Hegedus, 1994: 188, fig. 714 [misidentification].
Antillophos chazalici (Dautzenberg, 1900).—Merlano and Hegedus, 1994: 188, fig. 715; Watters, 2008: 5, figs. 6, 7; García, 2008b: 4, fig. 15.
Antillophos elegans (Guppy, 1866).—Petuch, 1987: 89, pl. 24, figs. 9, 10; Merlano and Hegedus, 1994: 188, fig. 717 [misidentification].

Description: Average 12.7 mm in length (min, 9.6; max, 24.0 [exceptional]). Fusiform; spire ca. 50–60% of total length. Protoconch minute, tan, conical, of ea. 2.25 smooth whorls with sharp keel at periphery; first whorl is sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 5.5 whorls. Teleoconch whorls sculptured with widely spaeed 1° spiral cords, ca. 12 on last whorl, excluding varix. 2° spiral sculpture of single thread present between 1° cords. Axial sculpture of widely spaced, low, rounded ribs separated by eoneave spaces; ca. 15 ribs on last whorl and 8–12 ribs on penultimate whorl. Previous varices very low or not differentiated from axial sculpture. Terminal varix low, thick, very wide, crossed by numerous axial ribs. Intersections of axial and spiral sculpture form posterior-pointing serrations. Aperture elongate-oval, with 2 plications at siphonal canal, anal canal set off by two weak denticles. Outer lip with 12–14 lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" small and shallow. Colored off-white with tan bands below suture, below periphery,



Figures 86–100. *Antillophos chazalici* (Dautzenberg, 1900). **86–87.** Holotype, Institute royal des Sciences naturelles de Belgique, unnumbered, 12 mm, photo courtesy Th. Hubin RBINS. **88–89.** GTW 4331g, 40 m, Isla Los Monjes, Colombia, 16.7 mm. **90.** GTW 4331f, 160 m, off La Romana, Dominican Republic, 14.7 mm. **91.** UF 186254, SW of Sombrero Key Light, Monroe Co., Florida, 16.6 mm. **92.** UF 164326, 100 m, Punta Jiguero, Puerto Rico, 12.8 mm. **93–94.** UF 381597, 53 m, 20.84° N, 92.32° W, Campeche, Mexico, 14.6 mm. **95.** HGL, 250–300 m, off Isla Escudo de Veragnas, Panama, 14.3 mm. **96.** HGL, 71–74 m, off Louisiana, 15.1 mm. **97–98.** GTW 13749a, 37–46 m, Islas Los Testigos, Venezuela, 15.3 mm. **99–100.** GTW 4331d, 43 m, Punta Espada, La Guajira, Colombia, 14.4 mm.

and on siphonal canal; bands darkest on varices. Aperture white. Operculum leaf-shaped, pale yellow, with anterior terminal nucleus. Radula and anatomy unknown. García (2008b: fig. 15) illustrated a living specimen.

Holotype: Institute royal des Sciences naturelles de Belgique.

Type Locality: Iles Testigos, [*Chazalie*] Stn. 26; Santa Marta, Stns. 42 et 44 [Venezuela]. It is not clear from which of the two localities the holotype originated.

Other Material Examined: Florida. UF 203973, 150 m, off Breakers, Triton Sta. 131, Palm Beach, Palm Beach Co.; UF 204115, 120 m, off Breakers, Triton Sta. 111 and 112, Palm Beach, Palm Beach Co.; UF 222924, 150 m, off Breakers, Triton Sta. 188-191, Palm Beach, Palm Beach Co.; UF 221840, 60 m, S of Palm Beach Pier, Triton Sta. 392-394, Palm Beach Co.; UF 205502, 225 m, off Palm Beach, Triton Sta. 18 and 19, Palm Beach Co.; UF 266956, 240 m, off Hillsboro Beach, Broward Co.; UF 425827, 130 m, Egmont Key, Tampa, Hillsborough Co.; FMNH 249643, 80 m, SW of Sombrero Light, Monroe Co.; UF 186254, SW of Sombrero Light, Monroe Co.; GTW 4331c, 60–75 m, W of Cedar Keys, Monroe Co. Louisiana. HGL, 71–74 m, 28°03′ N, 92°27′ W; UF 381550, EFG 26675, both 86–91 m, 28°01′ N, 92°28′ W; EFG 23207, 89–92 m, 28°07′ N, 90°58′ W; EFG 24384, 99.3 m, 28°06′ N, 91°02′ W; EFG 25054, wreck of the tanker Halo, 80 km off SW Pass, 28°17′ N, 89°58′ W. Mexico. UF 381634, EFG 26122, both 93–94 m, 20°51′ N, 92°26′ W, Campeche; UF 381597, EFG 26107, both 53 m, 20°50′ N, 92°19′ W, Campeche; UF 381598, 52–53 m, 20°46′ N, 92°13′ W, Campeche; UF 381582, 73–77 m, 20°00′ N, 92°26′ W, Campeche; EFG 26017, 107–108 m, 22°16′ N, 91°30′ W, Campeche. Panama. HGL, 50 m, algae, Panama; HGL, 250–300 m, off Isla Escudo de Veraguas; HGL, 250 m, mud, San Blas Islands; GTW 4331h, 120 m, San Blas Islands; GTW 4331e, 130 m, San Blas Islands. Cuba. UF 425821, Guamuhaya, Sancti Spíritus Province. Dominican Republic. GTW 4331f, 160 m, muddy bottom, off La Romana. Puerto Rico. UF 164325, 164326, both 100 m, Punta Jiguero. Venezuela. GTW 13749a, 37–46 m, Islas Los Testigos. Colombia. Phil Fallon coll. 10210090, 40 m, Punta Espada, La Guajira, Colombia; GTW 4331d, 43 m, trawled, Punta Espada, Guajira Peninsula; HGL, 60–80 m, Guajira Peninsula; GTW 4331g, Phil Fallon coll. 10402120, both 40 m, trawled, Islas los Monjes; HGL, 67 m, Cabo de La Vela; GTW 13749b, 60–80 m, Cabo de La Vela.

Distribution: Widely but sporadically recorded from the Gulf of Mexico and Caribbean Sea.

Habitat: Dead shells are found at depths of 40–240 m; live specimens are known from 50–200 m. Fresh-dead specimens have been dredged in mud. García (2008b: 4) described its habitat on the pinnacles off Louisiana as "a combination of sediment and rubble, as well as in finer sediment at the edge of pinnacles."

Etymology: Named after the yacht Chazalie, the research ship that dredged the type material.

Discussion: This is the smallest species of the western Atlantic *Antillophos* and has largely been forgotten. It resembles a miniature *A. candeanus* or *A. virginiae* and has one fewer whorl as an adult than those species (5.5 vs. 6.5), but possesses a characteristic wide terminal varix. Neither *A. candeanus* nor *A. virginiae* have any previous varix at 5.5 whorls, whether a terminal varix or not. Dall (1889: 179) recognized this species prior to its description by Dautzenberg: "There is a small variety of [*candeanus*] which is brighter colored and more finely sculptured..." but he ultimately considered it only a variety of *candeanus*. See Table 3 for a comparison with other species.

Antillophos oxyglyptus (Dall and Simpson, 1901)
(Figures 43–47, 101)

Phos oxyglyptus Dall and Simpson, 1901: 401–402, pl. 57, fig. 18; Abbott, 1974: 220 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842]; Rios, 1994: 120, pl. 39, fig. 508 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842].

Antillophos virginiae (Schwengel, 1942).—Rios, 1970: 89, pl. 26, middle right [misidentification].

Antillophos candei (d'Orbigny, 1842).—Rios, 1975: 93, pl. 27, fig. 384; Rios, 1985: 101–102, pl. 35, fig. 444; Petuch, 1987: pl. 24, fig. 6; Rios, 1994: 120, pl. 39, fig. 508 [all misidentifications].

Antillophos oxiglyptus [*sic*] (Dall and Simpson, 1901).—Rios, 1975: 93 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842].

Antillophos bayeri Petuch, 1987: 102–103, pl. 24, figs. 4, 5; Kaicher, 1990: No. 5863; Merlano and Hegedus, 1994: 188, fig. 713.

Antillophos oxyglyptus (Dall and Simpson, 1901).—Watters, 2008: 5, fig. 2.

Description: Average 20.4 mm in length (min, 17.0; max, 24). Fusiform; spire ca. 50% of total length. Proto-

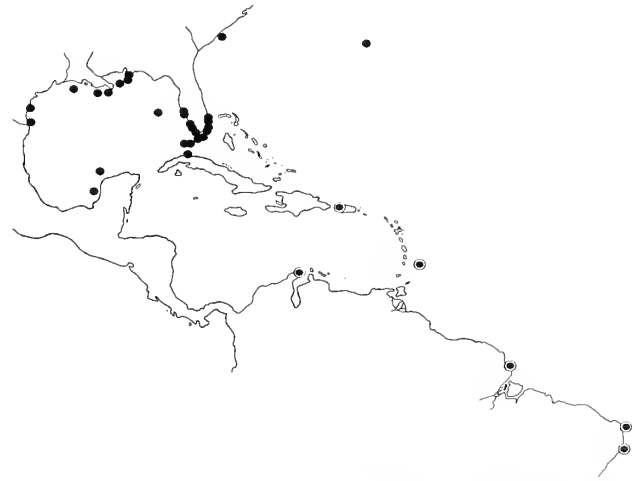


Figure 101. Distribution of *Antillophos oxyglyptus* (Dall and Simpson, 1901) (bullseye) and *Antillophos virginiae* (Schwengel, 1942) (solid).

conch minute, white, conical, of ca. 2.25 smooth whorls with sharp keel at periphery; first whorl is sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with narrow, widely spaced, flat, spiral cords separated by wide intervals, ca. 17 on last whorl. Interspaces with a single, fine, 2° spiral thread. Axial sculpture of widely spaced, low, rounded ribs separated by concave spaces; 16–18 ribs on last whorl (excluding varix) and ca. 16 on penultimate whorl. Varices well-developed, about one varix every 1/3 whorl except for last whorl. Terminal varix low, very wide, crossed by numerous axial ribs. Intersections of axial and spiral sculpture form pustulose sculpture. Aperture elongate-oval, with 2–5 denticles or plications anteriorly; anal canal set off by two denticles. Outer lip with 11–12 lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" small but deep. Colored white or off-white with three vague tan bands below suture, below periphery, and on siphonal canal, bands darkest on varices. Aperture white. Radula, operculum, and anatomy unknown.

Types: *Phos oxyglyptus* Dall and Simpson, 1901, holotype USNM 159696, listed as USNM 159676 in Boss et al. (1968) in error (*fide* Y. Villacampa, pers. comm., USNM, 2008); *Antillophos bayeri* Petuch, 1987, holotype USNM 859854.

Type Locality: (*oxyglyptus*) Mayaguez, Porto Rico. No bathymetric information was given; (*bayeri*) trawled by commercial shrimp trawler from 35 m depth off Cabo [de] La Vela, Guajira Peninsula, Colombia.

Paratypes: *Phos oxyglyptus* Dall and Simpson, 1901, a second specimen was indicated in the original description but has not been located; *Antillophos bayeri* Petuch, 1987, 1 shell, Robert Pace collection.

Other Material Examined: Barbados. HGL, GTW 9216a, 167–200 m, both dredged, silt, sand, coral rubble, 3.2 km W of Sandy Lane Bay, St. James. Colombia. USNM 859854, 35 m depth off Cabo de La Vela, Guajira Peninsula [holotype of *bayeri*].

Distribution: The actual range of this rare species is difficult to determine based on the scarcity of material, all dead shells, but it occurs at least from Puerto Rico to the Caribbean coast of Colombia and offshore to Barbados. Rios (1970, 1975) listed this species (as *A. virginiae* in 1970, as *A. candei* in 1975) from several locations off NE Brazil from Amapá to Alagoas states.

Habitat: Dead shells have been found in depths from 35–200 m on silt, sand, and coral rubble; Rios (1970) reported it from 60–80 m on a calcareous algal substrate.

Etymology: Gr. *oxys*, sharp + Gr. *glyptos*, carved.

Discussion: The type specimen is a faded, small individual, but clearly depicts the columellar denticles characteristic of this species. This species is very similar to the Gulf of Mexico species *Antillophos virginiae* in sculpture and apertural features. It has fewer axial ribs on the penultimate whorl than *A. virginiae* (16 vs. 20) and (so far) is separated from *A. virginiae* by a considerable distance. As with *A. virginiae*, the number and strength of the columellar denticles varies considerably. See Table 3 for a comparison with other species.

Antillophos smithi (Watson, 1885)
(Figures 48–55, 102)

Phos smithi Watson, 1885: 221, pl. 17, figs. 7a,b; Rios, 1975: 93, pl. 27, fig. 383; Rios, 1985: 102, pl. 35, fig. 445; Kaicher, 1990: No. 5871; Rios, 1994: 121, pl. 39, fig. 513.
Antillophos sp. Redfern, 2001: 92, pl. 43, figs. 390a, b.
Antillophos freemani Petuch, 2002: 64, 66, figs. 2c, d.
Antillophos smithi (Watson, 1885).—Watters, 2008: 5, fig. 8.

Description: Average size 28.3 mm in length (min, 21.7; max, 36.6). Fusiform; spire ca. 60% of total length. Protoconch minute, white, conical, of ca. 2.25 smooth whorls with sharp keel at periphery; first whorl is sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with flat, 1° spiral cords separated by incised grooves, ca. 16 cords on last whorl. 2° and occasional 3° spiral sculpture present in 1-2-3-1 or 1-2-1 pattern. Axial sculpture of widely spaced, low, rounded ribs separated by concave spaces; 13–18 ribs on last whorl, excluding varices, and 12–18 ribs on penultimate whorl. Previous varices present or absent, often one every ¾ whorl. Terminal varix narrow, set back a short distance from outer lip. Intersections of axial and spiral sculpture form low pustules. Aperture elongate-oval, with two weak plications at the siphonal canal; anal canal set off by two denticles. Outer lip with 14–21 fine lirae deep within mouth; lirae may be pustulose in some specimens. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" wide and shallow. Colored off-white with wide tan bands below suture, below periphery, and on siphonal canal; bands darkest on varices. Aperture white.

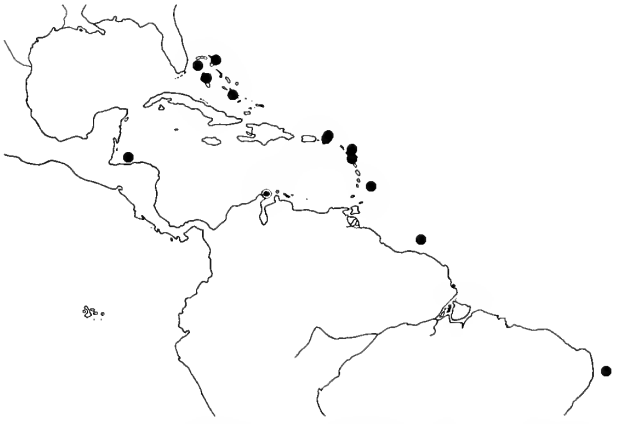


Figure 102. Distribution of *Antillophos smithi* (Watson, 1885) (solid) and *Antillophos verriculum* new species (bullseye).

Operculum leaf-shaped, yellow to dark brown, with anterior terminal nucleus. Radula and anatomy unknown.

Holotypes: *Phos smithi* Watson, 1885, BM(NH) 1887.2.9.751, 35 mm; *Antillophos freemani* Petuch, 2002, UF 277099, 25.6 mm.

Type Locality: (*smithi*) Sta. 221. Lat. 9°5′ S, long. 34°50′ W. off Pernambuco [State]. 350 fathoms [690 m]. Mud. [Brazil]; (*freemani*) [7 km SW] off Victory Cay, Bimini Chain, Bahamas.

Paratypes: *Antillophos freemani* Petuch, 2002, UF 277100, 3 shells, 25.6, 23.0, 19.6 mm; Petuch coll., 22 mm; Freeman coll., 26 mm; each 1 shell, all from the type locality.

Other Material Examined: Bahamas. UF 277099, 7 km SW of Victory Cay, Bimini; GTW 12864a, 250–300 m, off Great Guana Cay, Exumas; Bahamas; EFG 5358, 300 m, 26°49′ N, 77°01′ W; CR 4910, 13958, both 295 m, off Guana Cay, Abaco, 26°47′ N, 77°09′ W. Honduras. GTW 9163a, dredged 150 m, off Isla de Utila; GTW 9163b, baited traps, 230–260 m, off Roatán Island; HGL, 230–250 m, E Roatán Island..British Virgin Islands. GTW 9163c, fish pot at 7.5 m, Anegada; GTW 9163d, crabbed, 1 m, Soper's Hole, W end Tortola. Guadeloupe. UF 121214, 160 m, off Port Louis; MNHN, part of syntype lot of *A. beauii*, Marie-Galante. Barbados. HGL, 200–230 m, W of Barbados; HGL, 167–200 m, 3.3 km W Sandy Lane Bay, St. James. Surinam. EFG 5356, 236 m, 7°28′ N, 54°35′ W Colombia. EFG 19318, 280 m.

Distribution: There are scattered records of this species from the Bahamas throughout the Caribbean to Pernambuco State, Brazil. Specimens from Colombia have been sold to private collectors but the final disposition of these specimens is unknown.

Habitat: This is a fairly deep-water species with dead shells occurring mainly from 150–300 m (rarely crabbed from 7.5 m). Live specimens have been recorded from 150–260 m. Some have been collected in baited traps. Substrate unknown.

Etymology: "I have given it the name of Mr Edgar A. Smith whose ever kind help I have repeatedly had to appeal to" (Watson, 1886: 221). Smith was a contemporary of Watson at the British Museum.

Discussion: *Antillophos smithi* is based on a slightly immature type specimen. It is a rare, fairly deep-water species with a broad distribution but few records. It seems to have been forgotten by later writers. This large, handsome species is often referred to as "*Phos elegans* Guppy, 1866," a Miocene species. *Antillophos smithi* most closely resembles *A. beauii* in its large size and elongate shell, but differs in its pustulose sculpture in contrast to *A. beauii*'s polished surface. *Antillophos freemani* Petuch, 2002, is a pale, weakly sculptured variant from the Bahamas. Specimens from South America are more coarsely sculptured than more northerly populations. See Table 3 for a comparison with other species.

Antillophos verriculum new species
(Figures 102–107)

Antillophos candei (d'Orbigny, 1842).—Merlano and Hegedus, 1994: 188, fig. 714 [misidentification].
Antillophos sp.—Watters, 2008: 5, fig. 11.

Description: Shell 24.6–34.0 mm in length (holotype 31.6 mm in length). Fusiform; spire ca. 50 – 60% of total length. Protoconch minute, golden, conical, of ca. 2.25 smooth whorls with sharp keel at periphery; first whorl is sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with widely spaced, weak, narrow spiral cords, ca. 17 cords on last whorl. Axial sculpture of widely-spaced, low, rounded ribs, 9–17 ribs on last whorl (excluding varix) and ca. 16 ribs on penultimate whorl. Previous varices absent or scarcely differentiated from axial sculpture. Terminal varix low, very wide, with numerous axial ribs. Intersections of axial and spiral sculpture form posterior-pointing serrations. Aperture elongate-oval, with two weak plications at siphonal canal; anal canal delimited by weak denticles. Outer lip with ca. 15 lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" small but deep. Colored white with vague tan bands below suture, below periphery, and on siphonal canal, bands darkest on varices. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: UF 425835 (ex GTW).

Type Locality: 40 m, trawled, Punta Espada, Guajira Peninsula, Colombia.

Paratype: BMSM 17976, from the type locality (ex GTW).

Other Material Examined: Phil Fallon coll., 1 shell, from the type locality.

Distribution: Known only from the type locality.

Habitat: The type material, from 40 m, appears freshly dead. Substrate unknown.

Etymology: Latin *verriculum*, a seine, in reference to the texture of the sculpture; a neuter noun in apposition.

Discussion: This species is most similar to *Antillophos candeanus* but differs in having the axial ribs more serrate and farther apart and in having fewer lirations within the outer lip (11 vs. 15). None of the few specimens of *A. verriculum* have columellar denticles or lirae on the middle portion of the columella. *Antillophos verriculum* is a much thinner shell than *A. candeanus*, is more tabulate, and the aperture is more capacious. Most specimens have a rust-colored stain. See Table 3 for a comparison with other species.

Antillophos virginiae (Schwengel, 1942)
(Figures 101, 108–117)

Tritiaria (*Antillophos*) *virginiae* Schwengel, 1942: pl. 3, figs. 6, 7 [July], 65–66 [Oct.] [the captioned plate was published prior to the text description]; Abbott, 1974: 220 [in synonymy of *Cancellaria candcana* d'Orbigny, 1842]; Rios, 1985: 102 [in synonymy of *Cancellaria candcana* d'Orbigny, 1842]; Rios, 1994: 120 [in synonymy of *Cancellaria candeana* d'Orbigny, 1842].

Antillophos virginiea [*sic*] (Schwengel, 1942).—Rios, 1975: 93 [in synonymy of *Cancellaria candcana* d'Orbigny, 1842].

Antillophos candei (d'Orbigny, 1842).—Vokes and Vokes, 1983: 26, pl. 14, fig. 22 [misidentification].

Antillophos bayeri Petuch, 1987.—Robin, 2008: 182, fig. 5 [misidentification].

Antillophos virginiae (Schwengel, 1942). Watters, 2008: 5, figs. 9, 10; García, 2008b: 8, fig. 16.

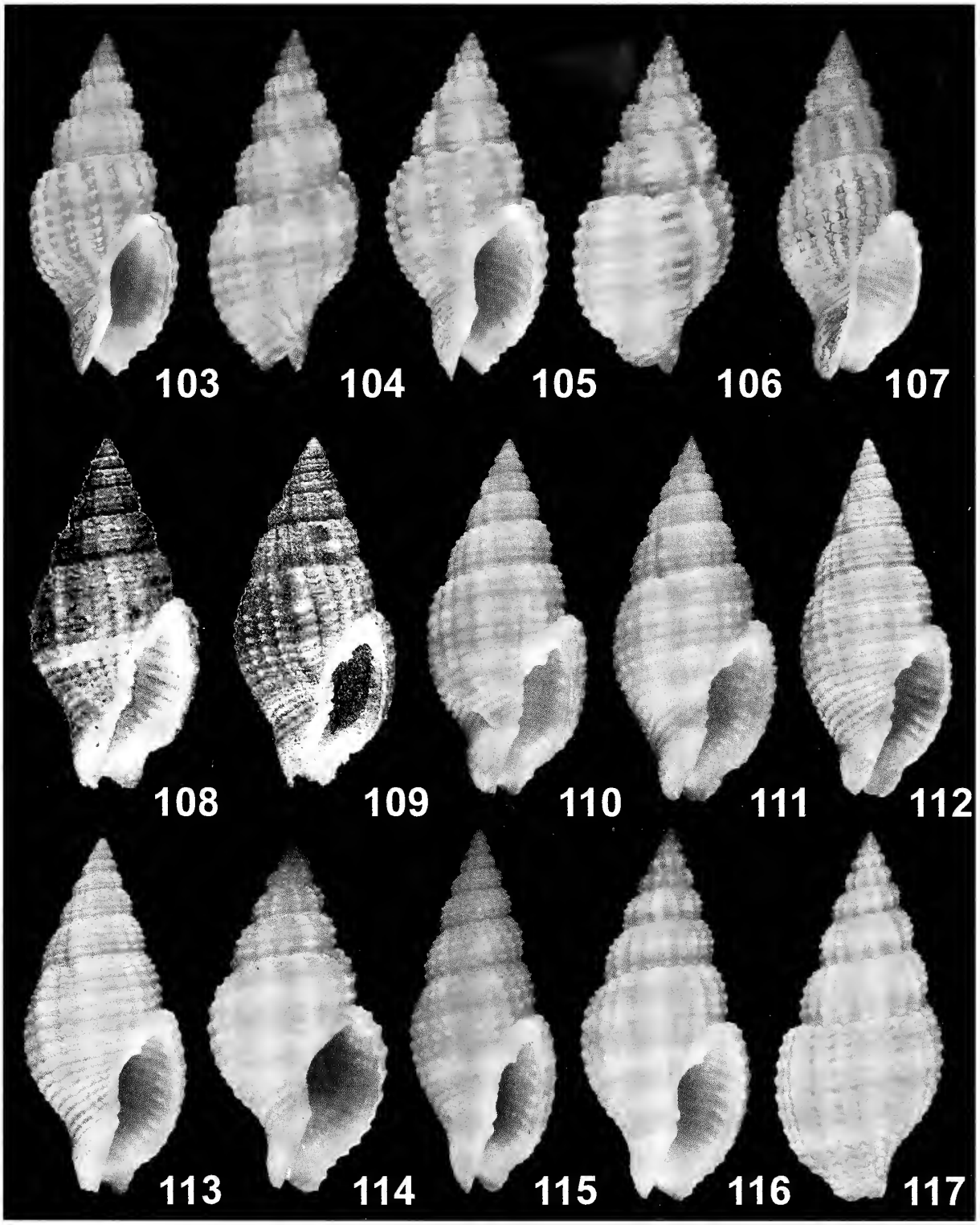
Description: Average 23.6 mm in length (min, 18.7; max, 32.2). Fusiform; spire ca. 50–60% of total length. Protoconch minute, white, conical, of ca. 2.25 smooth whorls with a sharp keel at periphery; first whorl is sunken into subsequent whorls; protoconch not distinctly delimited from teleoconch. Teleoconch of 6.5 whorls. Teleoconch whorls sculptured with widely spaced 1° spiral cords, ca. 15 cords on last whorl. 2° spiral sculpture of 2–4 threads between primaries. Axial sculpture of widely spaced, low, rounded ribs, ca. 14 ribs on last whorl, excluding varix, and 13–20 ribs on penultimate whorl. Previous varices very low, few. Terminal varix low, thick, very wide, crossed by numerous axial ribs. Intersections of axial and spiral sculpture form posterior-pointing serrations. Aperture elongate-oval, with 2–4 plications at the siphonal canal; anal canal with strong parietal tooth. Outer lip with 10–17 lirae deep within mouth. Columella continuous; parietal lip adherent to previous whorl. Siphonal canal short, open. "Stromboid notch" wide and shallow to deep. Colored uniformly white or with vague tan bands below suture, below periphery, and on siphonal canal, bands darkest on varices. Aperture white. Operculum leaf-shaped, yellow or reddish, with anterior terminal nucleus. Radula and anatomy unknown.

Holotype: ANSP 178716, lost (*fide* P. Callomon, pers. comm., 2008). Schwengel (1942) referred to a "type," but two specimens were illustrated on plate 3 (figs. 6, 7), neither identified as the holotype.

Type Locality: Dredged at 65 fms off Palm Beach, Florida.

Paratype: UF 150980, 133 m, off Palm Beach, Palm Beach Co., Florida, 1 shell, 25.9 mm; this is neither of the two specimens illustrated by Schwengel (1942).

Other Material Examined: Bermuda. HGL, 400 m, trapped, S of Castle Roads. South Carolina. GTW 4331i, 120 m, SE of Charleston, Charleston Co. Florida. UF 12725, 8 m, Lake Worth, Palm Beach Co.; UF 154749, 200 m, Lake Worth, Palm Beach Co.; UF 425822, 8 m, Lake Worth Inlet, Palm Beach Co.; UF 126248, 146480, 150980, 164329, 168057, 168088, 168206, 168225, 168239, 177142, 177583, 177671, 178946, 179066, 179128, 179504, 179513, 179686, 180089, 180114, 180116, 180187, 185350, 185460, 185947, 186313, 186331, 186365, 186379, 186395, 203368, 203462, 203692, 203950, 204163, 204194, 204297, 204316, 204363, 204558, 204572, 204654, 204658, 204666, 204762, 204790, 204842, 204987, 205132, 205337, 219603, 219780, 220007, 220056, 222080, 222174, 222827, 222890, 223163, 228485, 228819, 239643, 250711, 251100, 262207, 262249 (120–200 m), all Palm Beach, Palm Beach Co.; UF 222181, 120 m, off Lantana, Triton Sta. 169 and 170, Palm Beach Co.; UF 177277, 40–80 m, Manalapan to Lantana, Triton Sta. 520–523, Palm Beach Co.; UF 168557, 60–80 m, Lantana to Lake Worth Casino, Triton Sta. 381–384, Palm Beach Co.; UF 219933, 150–180 m, off Briny Breezes, Boynton Beach, Palm Beach Co.; UF 219827, 120 m, off McGinty house, Boynton Beach, Triton Sta. 364 and 365, Palm Beach Co.; UF 168179, 100–120 m, Boynton Inlet to Lantana Rd., Triton Sta. 378–380, Palm Beach Co.; UF 168159, 60 m, off Boynton Inlet, Palm Beach Co.; UF 168159, 60 m, off Boynton Inlet, Triton Sta. 377, Palm Beach Co.; UF 69985, 168576, 168593, 168659, 176313, 179107, 425825 (120–160 m), all off Delray Beach, Palm Beach Co.; UF 12723, off Hillsboro Light, Broward Co.; UF 266945, 160–200 m, off Hillsboro Beach, Broward Co.; UF 266946, 70–100 m, off Hillsboro Beach, Broward Co.; UF 127582, 80–140 m, off Pompano Beach, Broward Co.; BMSM 8101, 100 m, Miami Beach, Miami-Dade Co.; UF 126263, 54 m, E of Government Cut, Miami Beach, Miami-Dade Co.; UF 127144, Biscayne Bay, Miami-Dade Co.; UF 259115, 25–60 m, off Cape Sable, 25°09′ N, Monroe Co.; OSUM 3490, 116 m, St. Petersburg, Pinellas Co.; BMSM 8167, 40 m, W of Marco Island, Collier Co.; UF 259117, 50–60 m, off Cape Sable, 25°09′ N, Monroe Co.; UF 129869, 467+ m, 250 km W of Cape Romano, Collier Co.; UF 260326, 45–50 m, off Cape Romano, 25°40′ N, Collier Co.; UF 259381, 156 m, W of Ft. Myers, Lee Co.; UF 259343, 259344, both 210 m, W of Venice, Sarasota Co.; UF 122704, 400 m, off Tampa, Hillsborough Co.; UF 261531, 110 m, W of Tampa, Hillsborough Co.; UF 260805, 360 m, W of Tampa, Hillsborough Co.; UF 266950, 267 m, off Tampa, Hillsborough Co.; GTW 4331b, 300–400 m, W of Egmont Key, Tampa, Hillsborough Co.; UF 127806, 73 m, SW Egmont Key, Tampa, Hillsborough Co.; UF 239640, 140 m, SW Egmont Key, Tampa, Hillsborough Co.; HGL, 60 m, W of Egmont Key, Tampa, Hillsborough Co.; UF 126264, 130 m, Egmont Key, Tampa, Hillsborough Co.; UF 126247, 126255, 126257, 126259, 126261 (110–210 m), all 150° off Pensacola, Escambia Co.; UF 126250, 70 m, S of Pensacola, 29°25′ N, 87°20′ W, Escambia Co.; UF 266948, 250 m, off Key Largo, Monroe Co.; UF 126254, 165006, 165606, 165671, 165583, 165662, 165707, 165596, 168128, 256626, 168196, 168321, 168354, 168381, 168446, 168485, 168549, 176730, 180213, 185426, 185985, 186067, 186154, 186159,



Figures 103–117. *Antillophos* species. **103–107.** *Antillophos verriculum* new species. **103–104.** Holotype, UF 425835, 31.6 mm. **105–106.** Paratype, BMSM 17976, from the type loeality, 24.6 mm. **107.** Fallon coll. 10611020, from the type loeality, 34.0 mm. **108–117.** *Antillophos virginiae* (Schwengel, 1942). **108.** Schwengel (1942) figure 6. **109.** Schwengel (1942) figure 7. **110.** UF 239643, Palm Beach, Palm Beach Co., Florida, 25.9 mm. **111.** UF 125001, 233 m, SE of Alligator Reef Light, Monroe Co., Florida, 28.0 mm. **112.** UF 256771, 150 m, off Sand Key Light, Monroe Co., Florida, 30.5 mm. **113.** UF 266941, off Dry Tortugas, Florida, 21.5 mm. **114.** UF 219933, 120 m, off Briny Breezes, Boynton Beach, Palm Beach Co., Florida, 11.1 mm. **115.** HGL, 400 m, Bermuda, 32.2 mm. **116–117.** UF 142279, 84 m, S of Marquesas Keys, Florida, 22.5 mm.

186171, 186184, 186197, 186303, 186319, 186399, 186409, 228527, 239639, 250523, 256436, 256537, 256571, 256626, 256641, 256653, 256699, 258824, 377720 (80–250 m), all SW of Sombrero Key Light, Monroe Co.; UF 177118, 177849, 178003, 185418, 186143, 256407, 256440, 256450, 256771, 258648, 258664, 266947 (100–230 m) all off Sand Key Light, Monroe Co.; UF 165637, Archor, off Marathon, Monroe Co.; UF 185437, 256100, 266942, 185437, 256100, 266943, 266944 (140–250 m), all off Looe Key Reef, Monroe Co.; UF 266949, 120–140 m, off Grassy Key, Monroe Co.; UF 266951, 300 m, off Grassy Key, Monroe Co.; UF 125001, 233 m, SE of Alligator Reef Light, Monroe Co.; FMNH 259398, 169–200 m, Sand Key, off Key West, Monroe Co.; UF 122860, off Key West, Monroe Co.; UF 290012, Miller's Ledge, 24°26.965′ N, 82°09.156′ W, Monroe Co.; UF 36520, 110–117 m, 24°23′ N, 81°56′ W, Monroe Co.; UF 259382, 150 m, 29°12′ N, 85°50′ W, Monroe Co.; FMNH 170993, 100 m, off Dry Tortugas; BMSM 8100, 150 m, off Dry Tortugas; UF 266941, off Dry Tortugas; UF 197434, 238 m, W Dry Tortugas; UF 126251, 130 m, SE of Dry Tortugas; FMNH 194555, 91–213 m, SE of Dry Tortugas; UF 126258, 100 m, S of Marquesas Keys; UF 142275, 92 m, S of Marquesas Keys, 24°24′ N, 82°14′ W; UF 142267, 84 m, S of Marquesas Keys, 24°24′ N, 82°13′ W; UF 142279, 84 m, S of Marquesas Keys, 24°24′ N, 87°13′ W; UF 28772, 112 m, Straits of Florida, 24°24′ N, 82°02′ W; UF 29891, 128 m, Straits of Florida; BMSM 8166, Straits of Florida; EFG 13001, 140 m, 27°34′ N, 84°30′ W. Alabama. EFG 14451, 122 m, 29°14′ N, 88°15′ W; EFG 27724, 70–78 m, 29°34′ N, 87°59′ W; EFG 27702, 72–74 m, 29°24′ N, 87°59′ W. Louisiana. BMSM 38500, 28°05′ N, 91°00′ S; EFG 23207, 89–92 m, 28°07′ N, 90°58′ W; EFG 26674, 86–91 m, 28°01′ N, 92°28′ W; EFG 24395, 87.9 m, 28°05′ N, 91°00′ W. Texas. BMSM unnumbered, beach, Jefferson Co.; UF 266954, Port Aransas, Nueces Co.; UF 126262, 50 m, Port Isabel, Cameron Co. Mexico. UF 381623, BMSM 8664, Bay of Campeche; EFG 26121, both 93–94 m, Campeche, 20°51′ N, 92°26′ W; EFG 26016, 107–108 m, 22°16′ N, 91°30′ W. Cuba. UF 126260, 240 m, Bay of Matanzas, Matanzas Province. BMSM 8103, "western Atlantic."

Distribution: Known from Bermuda, South Carolina, south Florida, and the Gulf of Mexico. Dall's record (1889b) of "*Phos candei*" from Hatteras, North Carolina, is probably this species.

Habitat: In depths from 8 m (rare) to 450+ m. Dead shells are common off SE Florida in depths of 120–200 m; 27 specimens have been taken in a single sample. The few live individuals recorded were from 60–130 m. García (2008b: 8) recorded it from a mud bottom.

Etymology: Not stated, but probably named after Virginia Orr [Maes], malacologist contemporaneous with Schwengel at ANSP.

Discussion: This is the commonly dredged Floridian *Antillophos*. It is usually misidentified as "*candei*." Although true *A. candeanus* overlaps *A. virginiae* in south Florida (both have been found in the same sample off Palm Beach), and even occurs in somewhat shallower depths, *A. candeanus* is the much rarer of the two species in Florida. *Antillophos virginiae* is a south Florida and Gulf of Mexico species whereas *A. candeanus* is a Caribbean species unknown from the Gulf outside of southwest Florida. *Antillophos virginiae* is similar in shape and size to *A. candeanus* but differs in its much finer, pustulose sculpture compared to the coarse, serrate sculpture of *A. candeanus*. *Antillophos virginiae* usually has weak denticles or plications on the columella that are absent in *A. candeanus*. It is most similar to *A. oxyglyptus* from the Caribbean, which also may have columellar denticles. Schwengel referred her species to *Tritiaria*, a genus now believed to contain only fossil species (Haasl, 2000). The western Panamic cognate is *A. veraguensis* (Hinds, 1843). See Table 3 for a comparison with other species.

Genus *Bailya* M. Smith, 1944

Subgenus *Bailya* M. Smith, 1944

Bailya M. Smith, 1944: 78

Type Species: *Triton anomala* Hinds, 1844, by original designation.

Description: Small (to 17 mm in length). Fusiform; aperture 50–70% of shell length. Protoconch small, of 1.5 smooth, rounded whorls. Teleoconch sculpture of spiral threads and axial ribs; latter may be reduced on last ¼ whorl. Terminal varix is present. Aperture with weak denticles on outer lip. Columella smooth except for a denticle bounding anal canal, continuous, not angled at siphonal canal.

Discussion: Species of *Bailya* superficially resemble those of *Monostiolum* in overall shape and sculpture. However, the protoconch whorls of *Bailya* are rounded, whereas they are tabulate in *Monostiolum*. The columella is continuous in *Bailya* but distinctly angled at the siphonal canal in *Monostiolum*. See Table 1 for comparison with other genera.

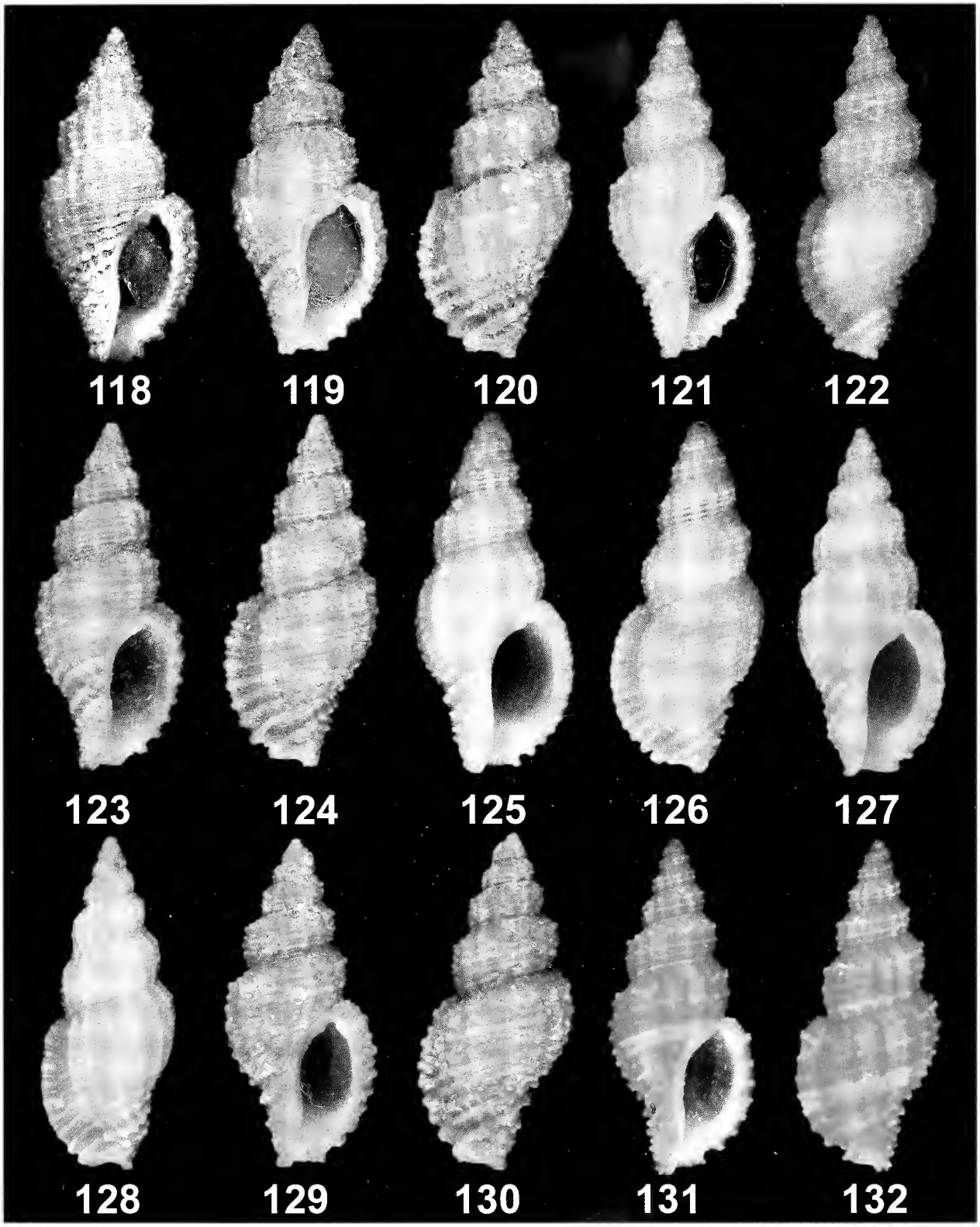
Faber (2004) named the genus *Steye* as a questionable buccinid with *S. janasaraiarum* Faber, 2004, as the type species. Faber compared the genus with *Bailya* but noted that the protoconchs are quite different. The protoconch of *Steye* is large, bulbous, with axial plications. It is quite distinct from any other buccinids in the western Atlantic if indeed it is a buccinid.

Bailya (Bailya) intricata (Dall, 1884)
(Figures 118–133)

Phos intricatus Dall, 1884: 325, pl. 10, fig. 9; Dall, 1889a: 58 [in synonymy of *Triton parvus* Adams, 1850]; Maury, 1922: 58 [in synonymy of *Triton parvus* Adams, 1850].

Phos parvus intricatus Dall, 1884. Olsson and Harbison, 1953: 260; Smith, 1936: 90.

Bailya intricata (Dall, 1884).—Abbott, 1954: 231, pl. 25t; Abbott, 1974: 217, fig. 2395; Kaicher, 1990: No. 5867.



Figures 118–132. *Bailya intricata* (Dall, 1884). **118.** Syntype, USNM 35961. **119–120.** GTW 5051b, 0.6 m, Bear Cut, Key Biscayne, Miami, Miami-Dade Co., Florida, 13.6 mm. **121–122.** GTW 5051g, 20 m, San Blas Islands, Panama, 15.1 mm. **123–124.** GTW 5051c, West Summerland Key, Monroe Co., Florida, 15.3 mm. **125–126.** GTW 5051d, 1 m, Punta Robles, Ambergris Cay, Belize, 13.0 mm. **127–128.** GTW 5051e, 2 m, Eleuthera, Bahamas, 12.5 mm. **129–130.** UF 70263, Havana, La Habana Province, Cuba, 15.8 mm. **131–132.** GTW 4257f, 8.3 m, Tambor Cay, Panama, 15.0 mm.

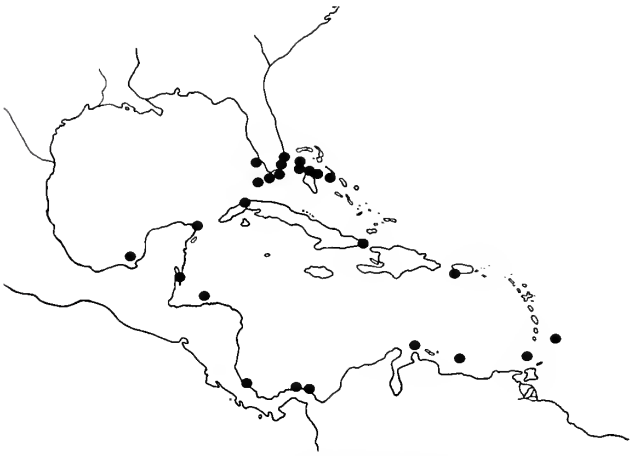


Figure 133. Distribution of *Bailya intricata* (Dall, 1884).

Bailya parva (Adams, 1850).—Vokes and Vokes, 1983: 25, pl. 14, fig. 12 [misidentification].
Bailya (*Bailya*) *intricata* (Dall, 1884). Watters, 2007: 10, figs. 1–3.

Description: Average 14 mm in length (min, 12.0; max, 16.5). Fusiform; spire ca. 50–60% total length. Protoconch blunt, of 1.5 smooth, rounded whorls. Teleoconch of 6 whorls, abruptly arising from protoconch. Teleoconch sculpture of 12–16 1° spiral threads, often bifid, on last whorl, including siphonal canal; cords distinctly raised and square in cross-section. Spiral cords on siphonal canal much stronger. 2° and 3° spiral cords also apparent, arranged in 1-3-2-3-1 pattern. Axial sculpture of widely-spaced, low ribs, 13–16 ribs on penultimate whorl, 13–16 ribs on last whorl. Intersections of axial and spiral sculpture form tuberculate lattice; tabulate below the suture. Sculpture strength varies considerably between populations. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, weakly crenulated on outer lip; anal canal set off by two denticles. Columella continuous, smooth. Parietal callus adherent to body whorl for its length. Siphonal canal short, open. Color dingy white or gray, occasionally with thin periostracum. Aperture white. Operculum leaf-shaped, yellow to nearly black, with anterior terminal nucleus. Radula and anatomy unknown.

Syntypes: USNM 35961, six shells.

Type Locality: Key West, Florida.

Other Material Examined: Florida. UF 266961, 80–120 m, off Hillsboro Beach, Broward Co.; UF 128071, 30 m, off Boynton Beach, Broward Co.; UF 157590, 20 m, Pompano Beach, Broward Co.; UF 70272, 205208, 250100, 250953, 261001, all Palm Beach, Palm Beach Co.; GTW 5051b, under rocks in 0.6 m, low tide, Bear Cut, Key Biscayne, Miami, Miami-Dade Co.; UF 145191, Venetian Causeway, Miami, Miami-Dade Co.; UF 266966, Ragged Rocks, Miami-Dade Co.; UF 47383, 10–80 m, off Miami, Miami-Dade Co.; UF 145203, 50 m, E of Government Cut, South Miami Beach, Miami-Dade Co.; UF 241404, Mashta Point, Key Biscayne, Miami, Miami-Dade Co.; FMNH 25902, 160611, 189448, UF 145207, 266968, all Bonefish Key, Florida Bay, Monroe Co.; FMNH 315154, 315162, both Lake Surprise, Key Largo, Monroe Co.; FMNH 21079, Garden Cove, Key Largo, Monroe Co.; UF 70271, 266965, Key Largo, Monroe Co.; FMNH 315222, Sand Island, near Molasses Reef, Key Largo, Monroe Co.; FMNH 315163, 7 m, Molasses Reef, Key Largo, Monroe Co.; UF 120741, 7 m, Pickles Reef, N Key Largo, Monroe Co.; BMSM 8129, Bahia Honda Key, Monroe Co.; GTW 5051c, West Summerland Key, Monroe Co.; FMNH 315168, 1 m, Raccoon Key, Monroe Co.; BMSM 8004, Summerland Key, Monroe Co.: FMNH 289306, UF 127145, 128059, 145201, 192109, 241405, all Ohio Key, Monroe Co.; FMNH 191362, 189386, UF 145193, 239648, 266963, all Missouri Key, Monroe Co.; UF 145187, Tea Table Key, Monroe Co.; FMNH 315172, 3 m, W side of small key E of Johnston Key, N of Sugarloaf Key, Monroe Co.; FMNH 315171, 3 m, Jeffrey Key, off NW Big Pine Key, Monroe Co.; BMSM 8002, 13 m, Lower Matecumbe Key, Monroe Co.; FMNH 315153, 1 m, Raccoon Key, Monroe Co.; UF 121776, Grassy Key, Monroe Co.; UF 266960, Grassy Key, Monroe Co.; FMNH 227523, Ohio Key, Monroe Co.; UF 123200, Middle Torch Key, Monroe Co.; UF 191402, Old Rhodes Key, Monroe Co.; FMNH 167030, Key West, Monroe Co.;UF 266967, N end of Key West, Monroe Co.; UF 394023, N end of Key West, Monroe Co.; UF 145202, Boca Grande Key, Monroe Co.; UF 12720, Boca Grande Reef, W Key West, Monroe Co.; UF 70131, 70132, 70273, 70275, 145192, 154785, all Key West, Monroe Co.; UF 145209, Middle Sambo Shoals, Key West, Monroe Co.; UF 239647, Sambo Reef, Key West, Monroe Co.; UF 145197, Washerwoman Shoals, near Key West, Monroe Co.; UF 145194, Pelican Shoals, Key West, Monroe Co.; UF 145196, Loggerhead Key, Dry Tortugas; FMNH 202942, UF 70270, 266969, all Dry Tortugas; UF 145188, 16 m, 220° off Naples, Collier Co.; BMSM 8571, FMNH 278920, both Florida Keys. Bahamas. UF 145199, Bimini; GTW 5051a, on reef at 12 m, E of Picquet Rocks, Bimini Islands; UF 145206, Chub Cay, Berry Islands; UF 145186, Morgan's Bluff, Andros; UF 145204, Delaport Point, New Providence; UF 145190, Clifton Point, New Providence; UF 145198, Nassau, New Providence; GTW 5051e, in sand, 2 m, Eleuthera. Cuba. UF 425819, Havana, La Habana Province; UF 266970, Jauco, Guantánamo Province. Puerto Rico. UF 164192, Puerto Rico; UF 145213, La Parguera. Barbados. UF 145208, Hastings Rocks. Grenada. UF 145181. Netherlands Antilles. UF 266974, Aruba. Venezuela. GTW 5051h, 3.3 m, Los Roques Island. Mexico. UF 361558, Cayos Arcas; UF 382278, Isla Contoy, 25 km N of Isla Mujares, Quintana Roo State. Costa Rica. UF 387542, Moín Bay, W of Portetc. Belize. GTW 5051d, Punta Robles, Ambergris Cay. Honduras. UF 383556, S side in Oak Ridge, Jonesville, and Caribe Point, Roatán Island. Panama. UF 145211, Isla Colón, Bocas del Toro Archipelago; UF 145212,

Almirante; UF 266962, Devil's Beach; UF 397106, Devil's Beach; UF 338529, Isla Payardi, Bahia las Minas; UF 266964, Isla Galeta; GTW 5051g, 20 m, San Blas Islands; GTW 4257f, 8.3 m, Tambor Cay.

Distribution: Widely distributed in southern Florida, throughout the Greater and Lesser Antilles, and from the Yucatan through Central and South America east to at least Tobago.

Habitat: It occurs subtidally to 120 m, but usually in much shallower water, often among coral rubble.

Etymology: Latin *intricatus*, entangled, probably referring to the fine, reticulate sculpture.

Discussion: This species is very similar to *Bailya parva* and some specimens may be difficult to differentiate, particularly along the Central American coast. Dall himself eventually (1889a) synonymized his species with *B. parva*. In general *B. intricata* has more axial ribs (10–14 on the penultimate whorl of *B. parva* vs. 13–16 in *B. intricata*) and is usually a uniform dingy white or grey whereas *B. parva* is white with one or more brown bands, although exceptions occur, particularly in Honduras. Florida specimens seem to be more coarsely sculptured than most populations. See Table 4 for a comparison with other species.

Bailya (Bailya) parva (Adams, 1850)
(Figures 134–149)

Triton parvus Adams, 1847: 228 [*nomen nudum*].
Triton parvus Adams, 1850: 59–60; Tryon, 1881: 28, 263 [in synonymy of *Triton eximeus* Reeve, 1846]; Clench and Turner, 1950: 322–323, pl. 40, fig. 12 [lectotype].
Phos parvus (Adams, 1850).—Dall, 1889a: 15, 180, 226; Dall, 1889b: 116–117, pl. 48, fig. 6; Dall and Simpson, 1901: 401; Maury, 1922: 58; Smith, 1936: 20.
Bailya parva (Adams, 1850).—Abbott, 1954: 231; Abbott, 1958: 72; Abbott, 1974: 217, fig. 2396; Humphrey, 1975: pl. 17, figs. 23, 23a; Redfern, 2001: 91, pl. 43, fig. 389.
Bailya intricata (Dall, 1884).—Vokes and Vokes, 1983: 25, pl. 14, fig. 12 [misidentification].
Bailya (Bailya) parva (Adams, 1850).—Watters, 2007: 10, figs. 4–7.
Bailya milleri (Usticke, 1959).—Robin, 2008: 183, fig. 12 [misidentification].

Description: Average 13.6 mm in length (min, 11.5; max, 17.1). Fusiform; spire ca. 50% total length. Protoconch blunt, of 1.5 smooth, rounded whorls. Teleoconch of 6 whorls, abruptly arising from protoconch. Teleoconch sculpture varies between populations. Spiral sculpture of some specimens consists of 10–12 1° spiral cords on last whorl, including siphonal canal, with single 2° and multiple 3° cords apparent between them and may become as large as 1° cords. Spiral cords on siphonal canal much stronger. Axial sculpture of widely spaced, low ribs, 10–14 ribs on penultimate whorl, 10–12 ribs on last whorl. Intersections of axial and spiral sculpture form tuberculate lattice in some specimens or low nodules in others. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, weakly crenulated on outer lip; anal canal set off by two denticles. Columella continuous, smooth. Parietal callus adherent to body whorl for its length. Siphonal canal short, open. Color white with tan bands at suture, periphery, and base; other populations brown with single basal white band. Aperture white. Operculum leaf-shaped, yellow, with anterior terminal nucleus. Radula with three-cusped central tooth and single lateral on each side with three cusps; outer cusp being largest. Radula illustrated in Pilsbry and Vanatta (1904: fig. 5) and redrawn in Watters and Finlay (1989: fig. 7a). Anatomy unknown.

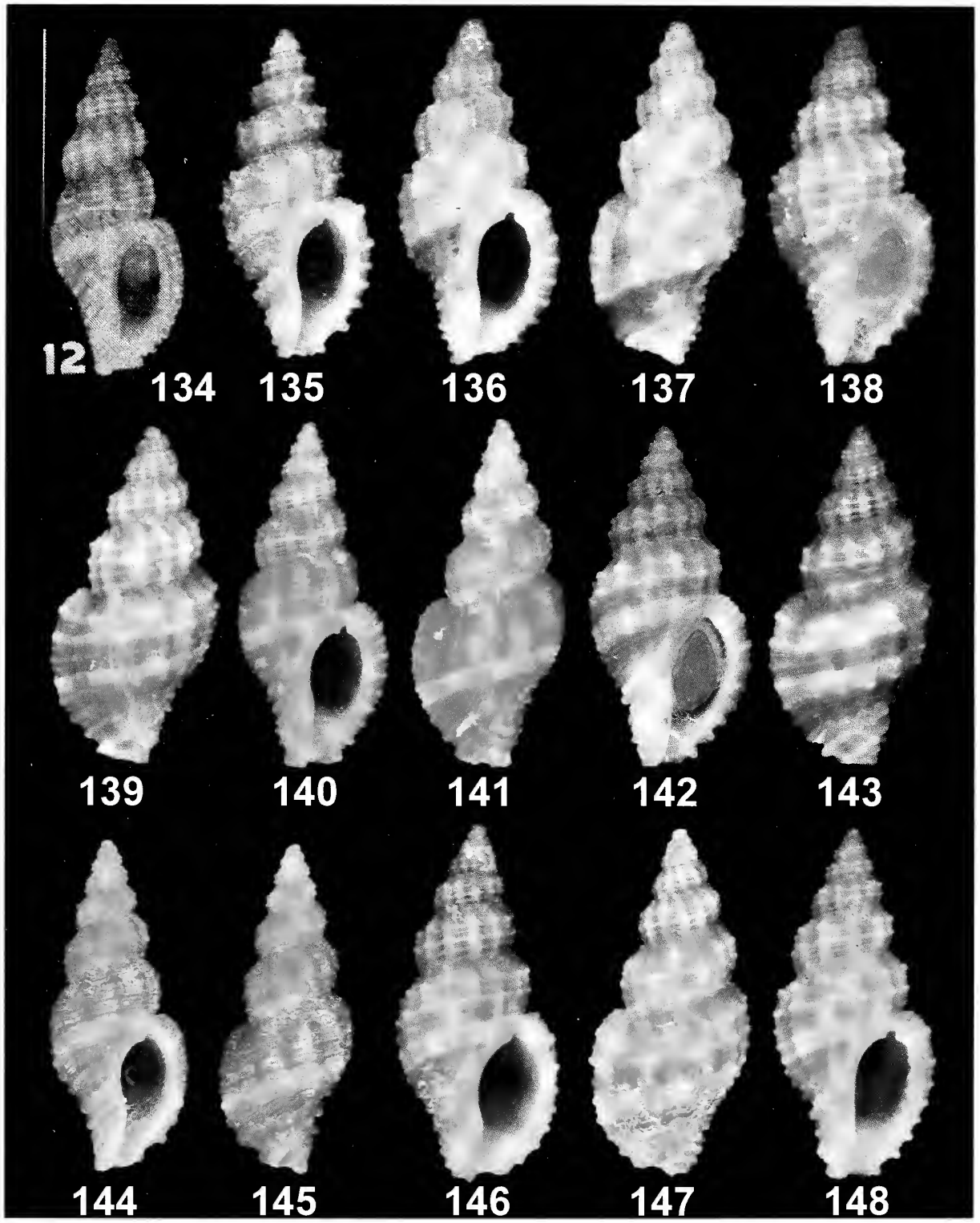
Lectotype: MCZ 177283, specimen not available for study but illustrated in Clench and Turner (1950), pl. 40, fig. 12, reproduced here.

Type Locality: Jamaica.

Other Material Examined: Florida. UF 145876, 205215, 228725, 250013, 250372, all Palm Beach, Palm Beach Co.; UF 145183, Yamato Rocks, Delray Beach, Palm Beach Co.; UF 191412, Elliot Key, Miami-Dade Co.; FMNH 289950, Key Largo, 25°03′ N, 80°29′ W; FMNH 289780, 0.3–1.6 m, Long Key Bight, ocean side of Long Key, Monroe Co.; BMSM 8625, Cudjoe Key, Monroe Co.; UF 25508, Garden Key, Dry Tortugas; FMNH 278920, Florida Keys. Bahamas. UF 145210, Gun Cay, Bimini; UF 266973, 398290, both Bimini; GTW 4257a, Bimini; UF 145189, Lyons Channel, North Bimini; UF 128054, South Bimini; UF 145205, Adelaide, New Providence; UF 145200, Clifton Bluff, New Providence; UF 145195, Nassau, New Providence; UF 121842, New Providence; UF 38210, Delaport, New Providence; UF 70274, New Providence; UF 267262, Grand Bahama Island; GTW 4257c, under rocks,

Table 4. Shell characteristics of *Bailya* species.

	Average length (max) mm	# spiral cords on last whorl	# axial ribs on last whorl	Color
intricata	14.0 (16.5)	12–16	13–16	dingy white (rarely banded)
morgani	12.2 (14.1)	15	obsolete (14–15)	white with brown subperipheral band
parva	13.6 (17.1)	10–12	10–13	white with brown peripheral, basal or sutural bands
sanctorum	8.3 (9.5)	17–25	obsolete (13–18)	white with brown subperipheral band
weberi	13.0 (16)	13–17	obsolete (11–14)	orange with white subperipheral band



Figures 134–148. *Bailya parva* (Adams, 1850). **134.** Lectotype, MCZ 177283, reproduced from Clench and Turner (1950), pl. 40, fig. 12. **135.** EFC 26246, 53 m, 20°50.22′ N, 92°18.91′ W, off Campeche, Mexico, 13.3 mm. **136–137.** GTW 4257h, 10–12 m, Cayos de San Andrés, Colombia, 16.9 mm. **138–139.** UF 70263, Havana, La Habana Province, Cuba, 11.4 mm. **140–141.** GTW 4257g, subtidal on reef, Negril, Jamaica, 13.1 mm. **142–143.** GTW 4257m, 10–12 m, St. Michiel to Spaanse Waters, Curaçao, Netherlands Antilles, 12.1 mm. **144–145.** EFC 25945, 46–48 m, 22°10′ N, 91°10′ W, off Campeche, Mexico, 12.5 mm. **146–147.** GTW 4257b, 1.3–3.3 m, under rubble, southern coast, Dominican Republic, 12.6 mm. **148.** GTW 4257c, 2.6–3.3 m, Tarpum Bay, Eleuthera, Bahamas, 13.1 mm.

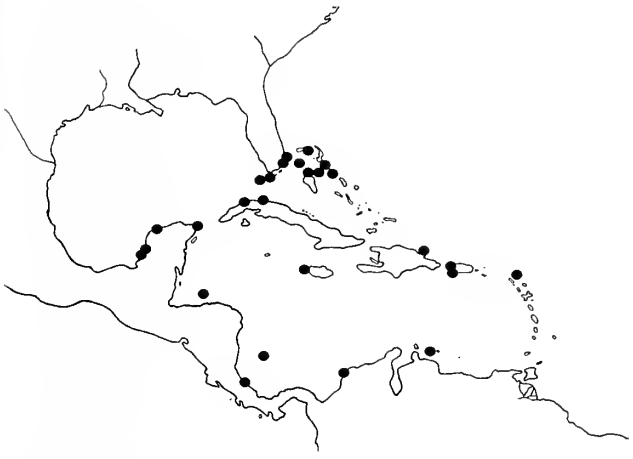


Figure 149. Distribution of *Bailya parva* (Adams, 1850).

2.6–3.3 m, Tarpum Bay, Eleuthera; UF 145184, Pigeon Cay, Andros; UF 145185, NW Athol Island; UF 267263, Harbour Island. Cuba. UF 70263, Havana, La Habana Province; UF 145177, Punta Hicacos, Varadero, Matanzas Province; UF 145178, Camarioca Reef, Matanzas Province. Jamaica. GTW 4257g, subtidal on reef, Negril. Dominican Republic. UF 353748, 6 km E of Las Terrenas, N Samaná Peninsula; GTW 4257b, 1.3–3.3 m, under rubble, southern coast. Puerto Rico. UF 162511, Terremoto Reef, off La Parguera; UF 164193, Mona Island. Antigua. UF 145180. Mexico. EFG 26246, 53 m, 20°50′ N, 92°19′ W, off Campeche; EFG 25945, 46–48 m, 22°10′ N, 91°10′ W, off Campeche; UF 387978, Isla Cerritos, Campeche State; F 388008, 16 km SW of Champoton, Campeche State; UF 361562, small point 27 km SW of Champoton, Campeche State; UF 383283, ca. 15 km N of Campeche, Campeche State; UF 354402, Playa Bonita, 8 km S of Campeche, Campeche State; UF 263932, Dzilam de Bravo, Yucatan State. Costa Rica. UF 352866, Limón. Honduras. EFG 9354, Cayos Cochinos; GTW 4257k, under coral rubble, 0.3–1.7 m, E Lime Key, SE Roatán Island; GTW 4257i, in reef rubble, 2.0–2.7 m, West Bay, Roatán Island. Colombia. HGL, GTW 4257j, GTW 4257h, all under rocks at 10–12 m, Cayos de San Andrés; GTW 4257l, coral reef, 2 m, Boca Chica, Cartagena. Netherlands Antilles. GTW 4257m, 10–12 m, St. Michiel to Spaanse Waters, Curaçao.

Distribution: Essentially the same as *Bailya intricata*. This seems to be a rarer species than *intricata*, at least in Florida.

Habitat: It occurs subtidally to 150 m, often among coral rubble.

Etymology: Latin *parvus*, small. In its original combination of *Triton parvus*, Adams undoubtedly was calling attention to its small size in comparison with other "tritons" such as *Charonia*.

Discussion: See *Bailya intricata* for a comparison with that species. See Table 4 for a comparison with other species.

Subgenus *Parabailya* Watters and Finlay, 1989

Bailya (*Parabailya*) Watters and Finlay, 1989: 55; Vermeij, 2001: 296 [in synonymy of *Bailya*].

Type Species: *Caducifer* (*Monostiolum*) *weberi* Watters, 1983, by original designation.

Description: Differs from *Bailya* sensu stricto in lacking strong sculpture on the final ½ whorl; the axial sculpture is particularly obsolete.

Bailya (*Parabailya*) *morgani* new species
(Figures 150–154, 165)

Bailya sp.—Watters, 2007: 10, fig. 12.

Description: Shell 9.9–14.1 mm in length (holotype 14 mm in length, 5.9 mm in width). Fusiform; spire 5–60% total length. Protoconch blunt, of 1.5 smooth, rounded whorls. Teleoconch of 6 whorls, abruptly arising from protoconch. Teleoconch sculpture of ca. 15 widely spaced, 1° spiral threads, including siphonal canal, between which are minute 2° threads; on last ¼ whorl all threads become equal in strength. Spiral cords on siphonal canal much stronger and tuberculate. Axial sculpture of widely spaced, low ribs; 12–15 ribs on last whorl. Axial ribs barely perceptible on last 1/4 whorl. Intersections of axial and spiral sculpture weakly tuberculate. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, weakly crenulated on outer lip; anal canal set off by two denticles. Columella continuous, smooth. Parietal callus adherent to body whorl for its length. Siphonal canal short, open. Color cream with broad, tan bands at suture, periphery, and base. Aperture white with tan bands showing through shell. Operculum leaf-shaped, yellow, with anterior terminal nucleus. Radula and anatomy unknown.

Holotype: UF 425840 (ex GTW).

Type Locality: Intertidal rocks, Caribe Point, Roatán Island, Honduras.

Paratypes: BMSM 17979, 1 shell, 12.6 mm, under rocks, 1–2 m, Roatán Island, Honduras (ex GTW); UF 425841, 1 shell, 9.9 mm, juvenile, 2.7 m, Dixon Cave, Roatán Island, Honduras (ex GTW).

Other Material Examined: Honduras. GTW 4257k, 1 shell, 0.3–1.7 m, E Lime Key, Roatán Island; EFG 5952, 10 shells, Caribe Point, Roatán Island; HGL, 1 shell, 0.6–1.3 m, E end Utila Island.

Distribution: Known only from Roatán and Utila Islands, Honduras.

Habitat: Only freshly dead and crabbed shells have been found, under intertidal rocks and coral rubble to 3 m.

Etymology: Named for the entrepreneurial Admiral Sir Henry Morgan (1635–1688), Welsh privateer, who had a base of operations at Port Royal on Roatán Island "employing" perhaps 5,000 people.

Discussion: This taxon appears to be endemic to Roatán and Utila Islands. However, Roatán and the other Bay Islands are known to harbor molluscs found no where else, including members of the Muricidae, Volutidae, and Turridae. *Bailya morgani* is similar to *B. sanctorum* new species (below) from the Virgin Islands but differs in its larger size, its geographic isolation, its higher spire, and in having coarser and less numerous axial ribs. It also resembles *B. intricata* but is less tabulate, less strongly sculptured, and with a different color pattern. *Bailya parva* has fewer and stronger axial ribs and usually a shorter spire. From the more widespread *B. weberi* it differs in coloration. See that species for comparison. See Table 4 for a comparison with other species.

Bailya (*Parabailya*) *sanctorum* new species
(Figures 155–159, 165)

Description: Shell 7.7–9.5 mm in length (holotype 9.5 mm in length, 4.5 mm in width). Fusiform; spire 50–60% total length. Protoconch blunt, tan or white, of 1.5 smooth, rounded whorls. Teleoconch of 6 whorls, abruptly arising from protoconch. Suture indented. Teleoconch sculpture of ca. 25 spiral threads on last whorl, including siphonal canal. Spiral cords on siphonal canal much stronger. Axial sculpture of widely-spaced, low ribs, 17–25 ribs on last whorl, 13–18 obsolete ribs on the penultimate whorl. Axial ribs barely perceptible on last ½ whorl. Intersections of axial and spiral sculpture weakly tuberculate. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, weakly crenulated on outer lip forming low lirate teeth; anal canal set off by two denticles. Columella continuous, smooth. Parietal callus adherent to body whorl for its length. Siphonal canal short, open. Color cream with broad tan band at base. Aperture white with tan band showing through shell. Operculum oval, orangish-tan, with an anterior terminal nucleus. Radula and anatomy unknown.

Holotype: UF 145179.

Type Locality: Trunk Bay, Saint John Island, US Virgin Islands.

Paratypes: UF 145179, 7.8 mm; UF 145179, 7.7 mm; from the type locality.

Distribution: Known only from the type locality. The paratypes were live-taken.

Habitat: No depth or substrate information is available.

Etymology: Latin *sanctorum*, of the saints. Named for the numerous Catholic saints lending their names to localities in the region: Saint Thomas, Saint John, Saint Croix, and the Virgin, as well as the fact that the type locality is a preserve, an ecological "holy place."

Discussion: This appears to be an endemic species, but how endemic remains to be seen. It is so far only known from the type locality, now part of the Virgin Islands National Park where collecting shells is forbidden, which may explain the dearth of records for this species. It differs from all other *Bailya* in its coloration, its diminutive size (being only ½–2/3 the size of other *Bailya* species), its stocky outline, and in having finer and more numerous axial ribs. It is the smallest *Bailya* known. See Table 4 for a comparison with other species.

Bailya (*Parabailya*) *weberi* (Watters, 1983)
(Figures 160–165)

Caducifer (*Monostiolum*) *weberi* Watters, 1983: 125–128, figs. 1–6, 11.

Bailya parva (Adams, 1850).—Sarasua and Espinosa, 1984: 6–7, fig. 4b [misidentification].

Monostiolum weberi (Watters, 1983).—Kaicher, 1987: No. 4856.

Bailya (*Parabailya*) *weberi* (Watters, 1983).—Watters and Finlay, 1989: 55–56, figs. 5e, f; Watters, 2007: 10, figs. 10, 11.

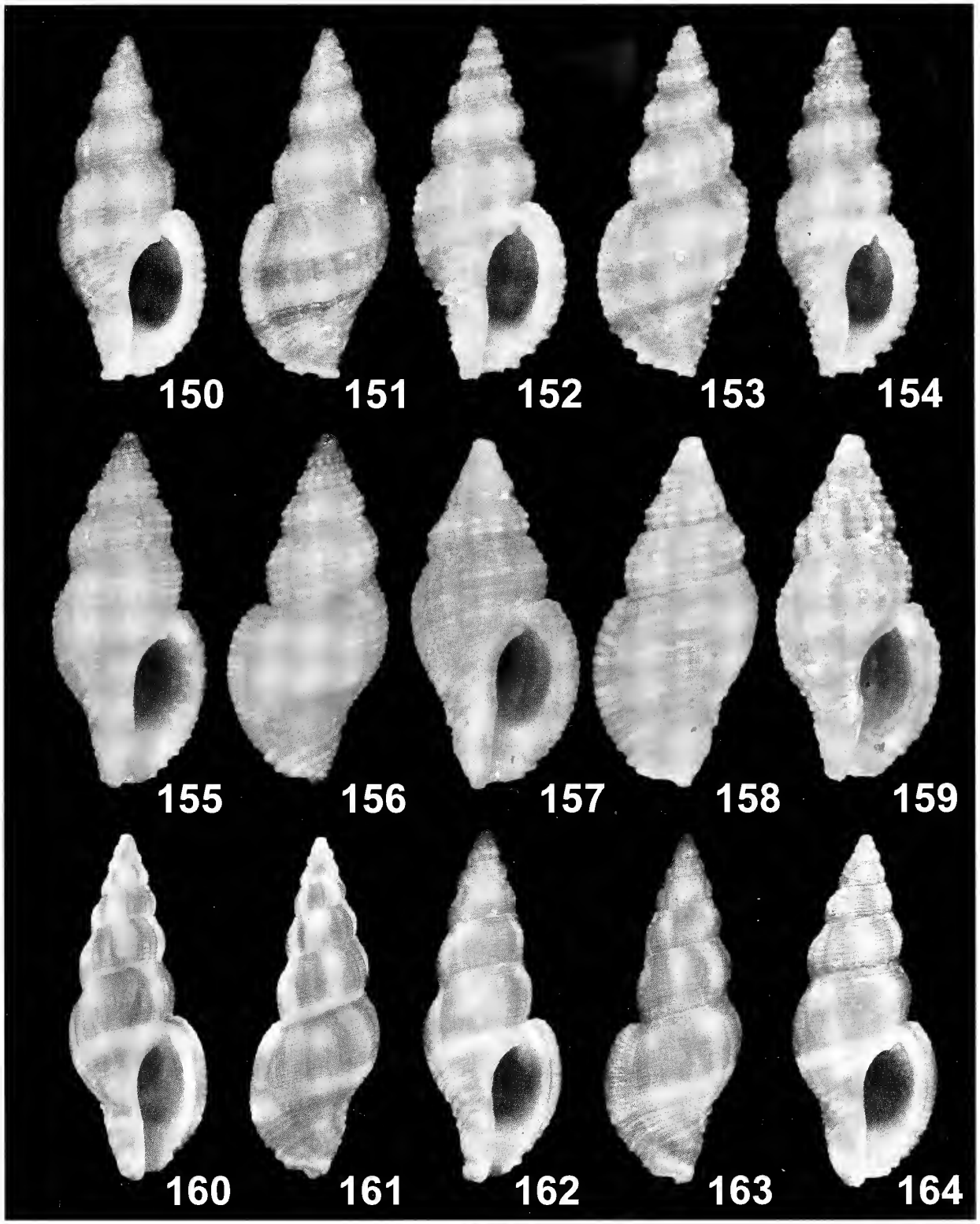
Description: Average 13.0 mm in length (min, 10.0; max, 16). Fusiform; spire ca. 60% total length. Protoconch blunt, of 1.5 smooth, rounded whorls. Teleoconch of ca. 7 whorls, abruptly arising from protoconch. Teleoconch sculpture of spiral cords, which may be bifid, separated by grooves of equal width; 13–17 cords on final whorl. Spiral cords become more subdued by sixth whorl; 2° and 3° threads appear in their interstices. Spiral threads more pronounced on siphonal canal. Axial ribs low, rounded, becoming less pronounced and irregularly spaced on later whorls, barely perceptible on the last ½ whorl; 12–14 ribs on penultimate whorl, 11–14 ribs on last whorl. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, weakly crenulated on outer lip; anal canal set off by two denticles. Columella continuous, smooth. Parietal callus adherent to body whorl for its length. Siphonal canal short, open. Color orange-brown, protoconch and occasional axial ribs white, with prominent, uninterrupted, white, subperipheral band. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: ANSP 355365, 16 mm.

Type Locality: 73 m off of Looe Key Reef, Big Pine Key, Monroe County, Florida.

Paratypes: AMNH 206077, USNM 617392, each 1 shell, La Chorrera sands, Havana, La Habana Province, Cuba.

Other Material Examined. Cuba. GTW 6830b, 6.7 m, María la Gorda, Pinar del Rio Province; UF 425820, Havana, La Habana Province; UF 57512, Havana, La Habana Province; UF 214438, La Chorrera sands, Havana, La Habana Province; UF 145227, 145228, both 20 m, La Chorrera sands, Havana, La Habana Province; UF 266972, Matanzas, Matanzas Province; UF 214437, Varadero, Matanzas Province; UF 298135, Varadero, Matanzas Province. Cayman Islands. UF 28938, Pirates Point Lodge, 1.2 km W of Airport, Little Cayman Island. Dominican Republic. GTW 6830a, under rubble, 4–6 m,



Figures 150–164. *Bailya* species. **150–154.** *Bailya morgani* new species. **150–151.** Holotype, UF 425840, 14.1 mm. **152–153.** Paratype, BMSM 17979, 12.6 mm. **154.** GTW 4257k, 0.3–1.7 m, E Lime Key, Roatán Island, Honduras, 12.8 mm. **155–159.** *Bailya sanctorum* new species. **155–156.** Holotype, UF 145179, 9.5 mm. **157–158.** Paratype, UF 145179, from type locality, 7.8 mm. **159.** Paratype, UF 145179, from type locality, 7.7 mm. **160–164.** *Bailya weberi* (Watters, 1983). **160–161.** Holotype, ANSP 355365, 16 mm, photos courtesy of R. Bieler (FMNH). **162–163.** UF 214438, La Chorrera sands, Havana, La Habana Province, Cuba, 15.0 mm. **164.** UF 298135, Varadero, Matanzas Province, Cuba, 14.1 mm.

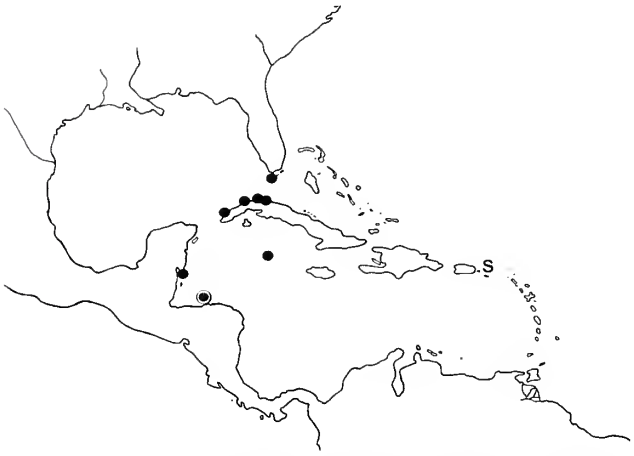


Figure 165. Distribution of *Bailya morgani* new species (bullseye), *Bailya sanctorum* new species (S), and *Bailya weberi* (Watters, 1983) (solid).

Cay Caulken reef. Mexico. UF 383438, Punta Honga, Quintana Roo State.

Distribution: *Bailya weberi* has a rather limited range in the western Atlantic Ocean: the Florida Keys, Yucatan, the Cayman Islands, Cuba, and Hispaniola. It is best known from La Chorrera sands off Havana. *Bailya intricata*, *B. parva*, and *B. weberi* have been taken in the same sample off Havana.

Habitat: Depth records place it between 4 and 73 m, probably in coral rubble, but these records are for dead shells.

Etymology: Named after the late Jay Weber of Miami, Florida, who assembled one of the largest private collections of his time. The holotype was derived from his collection.

Discussion: This brightly colored species cannot be confused with any other. Although a few specimens show some rugose ribs on the final whorl, the majority of specimens have very weak axial sculpture there. Two additional *Bailya* (*Parabailya*) are described here. *Bailya morgani* new species differs from *B. weberi* in its coloration, being cream with a brown sub-peripheral band; *B. weberi* is orange with a white sub-peripheral band; *B. sanctorum* new species is cream colored with a faint brown band and is less than 2/3 as large as *B. weberi*. See Table 4 for a comparison with other species.

Genus *Caducifer* Dall, 1904

Caducifer Dall, 1904: 136–137.

Type Species: *Triton truncatus* Hinds, 1844, by original designation.

Description: Overall very similar to *Monostiolum* (see below) but differs in being decollate as an adult. There are no appreciable differences between the western Atlantic species and those from the Pacific Ocean that would suggest that they do not belong to the same genus. While it could be argued that *Caducifer* is a subgenus of *Monostiolum*, the absence of *Monostiolum* in the Indo-West Pacific and the presence of *Caducifer* in both oceans suggests to me that the decollate state of *Caducifer* is an important characteristic at the genus level. The radula of *C. decollata* (Sowerby I, 1833) was illustrated by Ponder (1972: fig. 1.3). It differs from that of *Monostiolum tessellatum* in having the central tooth bearing five rather than three cusps. See Table 1 for comparison with other genera.

Caducifer atlanticus Coelho, Matthews and Cardoso, 1970 (Figures 166–173, 181)

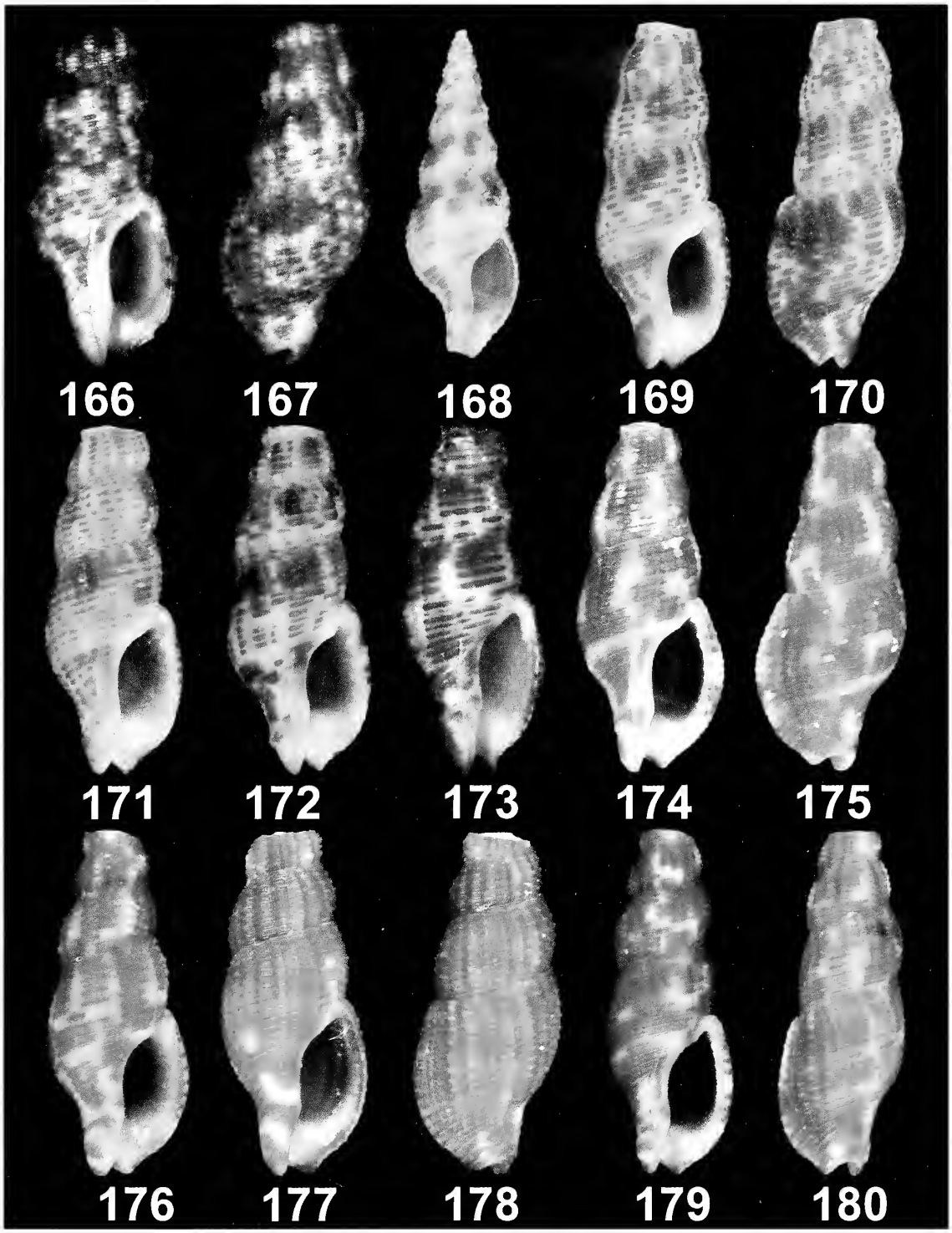
Caducifer atlanticus Coelho, Matthews and Cardoso, 1970: 185–188, figs. 1–3; Rios, 1975: 93–94, pl. 27, fig. 386; Rios, 1985: 99, pl. 34, fig. 436; Leal, 1991: 151, pl. 19, fig. E [protoconch]; Rios, 1994: 121, pl. 39, fig. 513.
Caducifer atlantica [*sic*] Coelho, Matthews and Cardoso, 1970.—Watters and Finlay, 1989: 57.

Description: Average size 13.6 mm in length (min, 13.0; max, 14.5), cylindrical, decollate. Fusiform; decollate spire ca. 60% total length. Protoconch blunt, of 1.25–2 smooth whorls, tabulated. First portion somewhat immersed in remaining part. Teleoconch of 3 whole and partial 4th whorl in decollate adult; 6.5 teleoconch whorls on only immature, non-decollate specimen seen. Spiral cords rounded; ca. 25 cords on last whorl, separated by wide flattened spaces crossed with microscopic threads. Axial ribs rounded, widely separated, C-shaped on final whorl; ca. 16 ribs on penultimate whorl, ca. 14 ribs on final whorl. Terminal varix well-developed, thickened. Aperture elongate, somewhat constricted. Parietal lip erect for much of its length, thickened. Anal canal bounded by weak thickening of columella and a denticle on inner lip. Inner lip with ca. 7 lirate teeth. Columella angled at siphonal canal. Siphonal canal short, open. Color white with dark brown, irregular sutural and siphonal canal patches. Spiral cords with brown areas on axial ribs. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: Museu Nacional, Brazil, MNRJ 3550, 13 mm.

Type Locality: Praia do Andrada, Trindade Island, Brazil.

Paratypes: LABOMAR, Instituto de Ciências do Mar, Universidade Federal do Ceará, 485, 1 shell, 60 m, Praia de Mucuripe, Fortaleza, Ceará State, Brazil; Museu Nacional, Brazil, MNRJ 3548, 1 shell, 60 m, Praia de Mucuripe, Fortaleza, Ceará State, Brazil; LABOMAR, 486, 1 shell, Trindade Island, Brazil; Museu Nacional, Brazil, 3549, 1 shell, Ilha da Trindade, Brazil; Museu de Zoologia da Universidade de São Paulo, 18506, 1 shell, Praia das Tartarugas, Trindade Island, Brazil; P.S. Cardoso coll., 3580 (Maceió), 1 shell, Praia do Principe, Trindade Island, Brazil; Museu Oceanográfico de Rio Grande, 15860, 1 shell, Praia da Enseada da Cachoeira, Trindade Island, Brazil; Museu Nacional, Brazil, 3551,



Figures 166–180. *Caducifer* species. **166–173.** *Caducifer atlanticus* Coelho, Matthews and Cardoso, 1970. **166–167.** Holotype, Museu Nacional, Brazil 3550, 13 mm, photos courtesy P. M. Costa (Museu Nacional, Brazil). **168.** GTW 10261g, 33 m, off Guarapari, Espírito Santo State, Brazil, 13.6 mm. **169–171.** GTW 10261c, 20–25 m, under rocks, off Rio do Fogo, Rio Grande do Norte State, Brazil. **169–170.** 13.8 mm. **171.** 13.0 mm. **172.** 30 m, under rocks, Cajueiro, Rio Grande do Norte State, Brazil, 14.5 mm. **173.** Paratype, Museu Naeional, Brazil 3548, size unknown, photo courtesy P.M. Costa (Museu Nacional, Brazil). **174–180.** *Caducifer camelopardalus* new species. **174–175.** Holotype, UF 425838, 11.4 mm. **176.** Paratype, UF 425839, from the type locality, 11.0 mm. **177–178.** Paratype, BMSM 17974, 110–140 m, off Cabo Frio, Rio de Janeiro State, Brazil, 11.2 mm. **179–180.** Paratype, OSUM 35444, 32 m, under roeks, off Porto Seguro, Bahía State, Brazil, 13.9 mm.

1 shell, Praia da Enseada da Cachoeira, Trindade Island, Brazil.

Other Material Examined: Brazil. GTW 10261f, 30 m, under rocks, Cajueiro, Rio Grande do Norte State; GTW 10261g, 33 m, under rocks, coral bottom, off Guarapari, Espírito Santo State; BMSM 17999, GTW 10261c, GTW 10261h, all 20–25 m, under rocks, off Rio do Fogo, Rio Grande do Norte State; HGL, beached, Ilha da Trindade, Espírito Santo State.

Distribution: Northeastern Brazil in Bahía, Ceará, Espírito Santo, Rio Grande do Norte, and Rio de Janeiro States, including Trindade Island and offshore seamounts of Vitória, Davis, and Dogaressa Seamounts (Leal, 1991).

Habitat: The specimens from 60 m were found in the "pacamon," a type of toadfish (*Amphichthys cryptocentrus* (Valenciennes, 1837)). Freshly dead shells have been recorded from 20–33 m under rocks on a coral bottom.

Etymology: From the Atlantic Ocean.

Discussion: See Table 5 for a comparison with *Caducifer camelopardalus* new species (below).

Caducifer camelopardalus new species
(Figures 174–181)

Description: Shell 11.1–13.8 mm in length (holotype 11.4 mm in length), decollate. Fusiform; decollate spire 50–60% total length. Protoconch unknown. Teleoconch of 3 whole and a partial fourth whorl in decollate adult. Spiral cords rounded, ca. 27 cords on last whorl, separated by wide flattened spaces crossed with microscopic threads. Axial ribs rounded, widely separated; ca. 17 ribs on penultimate whorl, ca. 18 weak ribs on final whorl. Terminal varix well-developed, rather narrow. Aperture elongate, not constricted. Parietal lip barely erect for much of its length, thickened. Anal canal bounded by very weak thickening of columella and a denticle on inner lip. Inner lip with ca. 9 weak, lirate teeth nearly absent in some specimens. Columella angled at siphonal canal. Siphonal canal short, open. Color white with orangish-tan blotches forming vague stripes and flammulations and a white subperipheral band; some specimens are almost uniformly dark, reddish brown. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: UF 425838 (ex GTW).

Type Locality: 20–25 m, under rocks, 70 km off Alcobaça, Bahia State, Brazil.

Paratypes: UF 425839, 1 shell, 11.0 mm, from the type locality (ex GTW); BMSM 17974, 1 shell, 11.2 mm, 110–140 m, off Cabo Frio, Rio de Janeiro State, Brazil (ex GTW); OSUM 35444, 1 shell, 13.9 mm, 32 m, under rocks, off Porto Seguro, Bahía State, Brazil (ex GTW).

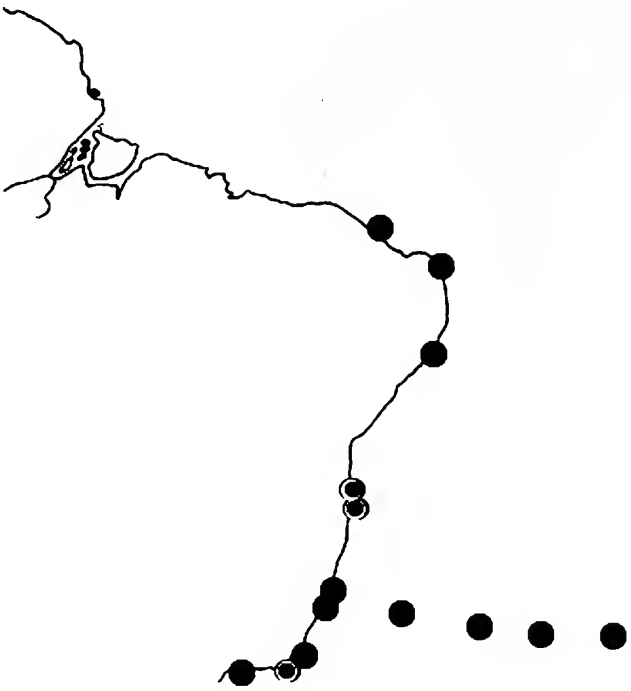


Figure 181. Distribution of *Caducifer atlanticus* Coelho, Matthews and Cardoso, 1970 (solid) and *Caducifer camelopardalus* new species (bullseye).

Other Material Examined: Brazil. GTW 10261e, 110–140 m, off Cabo Frio, Rio de Janeiro State.

Distribution: Off Bahía and Rio de Janeiro States, eastern Brazil; it has not been found on the seamounts where *C. atlanticus* occurs.

Habitat: Freshly dead shells have been found under rocks at 20–140 m.

Etymology: Latin *camelopardalus*, [spotted like] a giraffe.

Discussion: This species occurs within the range of *C. atlanticus* but may live in deeper water. *Caducifer camelopardalus* differs from *C. atlanticus* is having a narrow terminal varix (thicker in *C. atlanticus*), weak to absent denticles on the inner margin of the outer lip (denticles more developed in *C. atlanticus*), and a color pattern of large orangish blotches (small dark brown blotches and spiral lines in *C. atlanticus*). See Table 5. A very similar but undescribed species occurs at Escudo de Veraguas

Table 5. Shell characteristics of *Caducifer* species.

	Average length (max) mm	Inner lip	Varix	Color
atlanticus	13.6 (14.5)	Denticles well-developed	Thick	Dark brown patches and lines
camelopardalus	11.3 (13.8)	Denticles weak or absent	Narrow	Orange-brown flammulations

Island, Panama, but the disposition of the sole specimen, sold to a private collector, is unknown to me.

Dianthiphos new genus

Description: Fusiform; spire ca. 50% of length. Protoconch bulbous, 1.5 whorls, smooth, pink in the two known species. Teleoconch of 5 whorls, with spiral threads and axial ribs that become obsolete on last whorl. Single, thick, terminal varix. Columella angled at siphonal canal with a single denticle bounding anal canal. Outer lip without denticles, or with weak denticles bounding the anal canal. No internal lirae. Siphonal canal short, open.

Type Species: *Pisania bernardoi* Costa and Gomes, 1998.

Etymology: Latin *dianthus*, carnation, a pink, in reference to the pink protoconch.

Discussion: Costa and Gomes (1998) placed their species *bernardoi* in *Pisania* Bivona-Bernardi, 1832, a genus based on the European *P. striata* (Gmelin, 1791). Several western Atlantic species have been placed in *Pisania*, including *P. auritula* (Link, 1807) and *P. tincta* (Conrad, 1846), both now considered members of *Gemophos* Olsson and Harbison, 1953 (Vermeij, 2006), and *P. pusio* (Linnaeus, 1758). Both *P. striata* and *P. pusio* differ from *P. bernardoi* in having much larger shells, different protoconchs, incised spiral sculpture, columellar lirae (in *P. pusio*), and lirate outer lips. *Dianthiphos* differs from *Monostiolum*, conchologically the most similar genus in the western Atlantic, in its large, bulbous protoconch; the protoconch of *Monostiolum* is small, conical, and tabulate. *Antillophos* has a small, conical, keeled protoconch. *Bailya* has a small, rounded protoconch and a continuous columella, the latter of which is angled in *Dianthiphos*. See Table 1 for further comparison with other genera.

Dianthiphos is similar to several Indo-West Pacific genera. *Sukunaia* Cernohorsky, 1966, type species *S. jenningsi* Cernohorsky, 1966, also has a purple protoconch but lacks sculpture on the final whorls (corded in *Dianthiphos*) and has a denticulate outer lip (smooth in *Dianthiphos*). *Appisania* Thiele, 1929, type species *A. montrouzieri* (Crosse, 1862), also is denticulate. Nevertheless the three genera seem closely related. *Ecmanis* Gistel, 1848, type species *E. igneum* (Linnaeus, 1758), and *Taeniola* Dall, 1904, type species *T. decollata* (Sowerby, 1833), both differ from *Dianthiphos* in their smaller protoconchs and incised spiral sculpture.

Dianthiphos bernardoi (Costa and Gomes, 1998)
(Figures 182–185, 196)

Pisania bernardoi Costa and Gomes, 1998: 15–17, figs. 1–4; Robin, 2008: 193, fig. 6.

Description: Average size 15.2 mm in length (min, 12.4; max, 19.6). Fusiform; spire ca. 50% the total length. Protoconch bulbous, of 1.5 smooth, pink whorls. Teleoconch of 5 whorls, abruptly arising from protoconch. Teleoconch sculpture of 16–18 flattened, spiral threads, including siphonal canal, with intercalated 2° threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of closely spaced low ribs; 13–17 ribs on penultimate whorl, becoming obsolete on final whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, thick. Aperture oval, outer lip without teeth or with only weak denticles at anal canal. Columella angled at siphonal canal and bearing a weak denticle at anal canal and a single plication at siphonal canal; parietal lip adherent to previous whorl for all of its length. Siphonal canal short, open. Color brown to yellow with white blotches and white sub-peripheral band. Aperture white. Operculum leaf-shaped, yellow, with anterior terminal nucleus. Radula and anatomy unknown.

Holotype: Museu Oceanográfico Eliézer Rios da Fundação Universidade de Rio Grande, Brazil, MORG 39.006.

Type Locality: Continental slope off the coast of Salvador, Bahia State, Brazil.

Paratypes: Museu Nacional, Brazil, MNRJ 7163, off Guarapari, Espírito Santo State, Brazil; Museu de Zoologia de São Paulo, Brazil, MZSP 28.196, off Guarapari, Espírito Santo State, Brazil; "USNM, off Guarapari, Espírito Santo State, Brazil" (indicated in original description but stated paratype not in USNM collection); Muséum National d'Histoire Naturelle, Paris, off Guarapari, Espírito Santo State, Brazil; Insituto de Biologia da Universidade Federal do Rio de Janeiro, Brazil, IBUFRJ 6786, off Guarapari, Espírito Santo State, Brazil.

Other Material Examined: Brazil. GTW 9143a, HGL, both under rocks, 20–25 m, off Guarapari, Espírito Santo State; GTW 9143b, lobster nets, 50–60 m, off Guarapari, Espírito Santo State; GTW 9143c, among rocks, 1–3 m, Cabo Frio, Rio de Janeiro State.

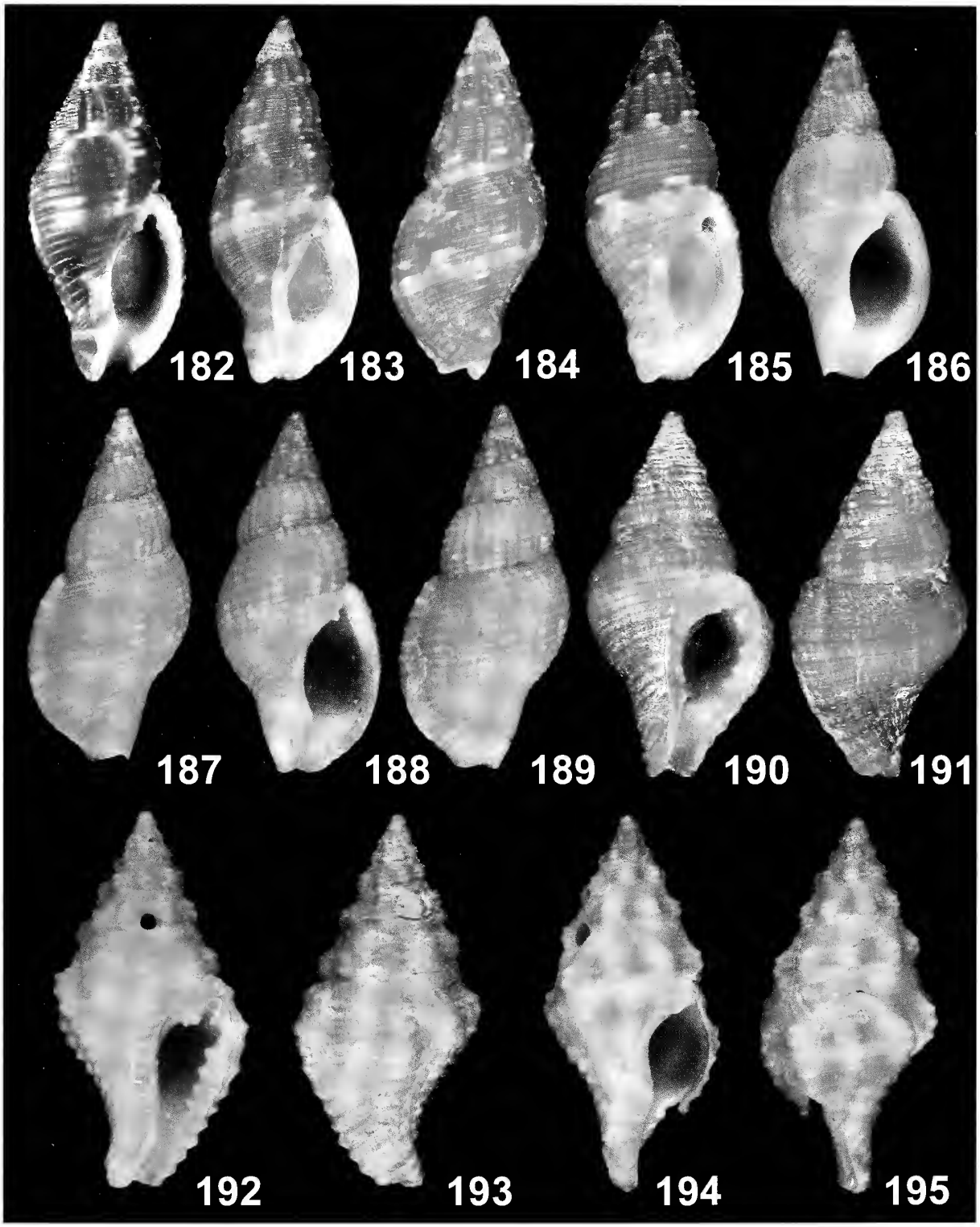
Distribution: Recorded from southern Espírito Santo State to Rio de Janeiro State, Brazil.

Habitat: Dead shells have been recorded from 1 to 60 m; live individuals are known from 20–25 m, under rubble.

Discussion: This species differs from the Colombian *D. electrum* new species by being slightly smaller, much more fusiform, much darker in color, and having fewer axial ribs on the penultimate whorl (ca. 16 in *bernardoi* vs. ca. 28 in *electrum*). It is geographically separated by ca. 4,600 km. See Table 6 for further comparison.

Dianthiphos electrum new species
(Figures 186–189, 196)

Description: Shell 15.9–16.7 mm in length (holotype 16.7 mm in length). Fusiform; spire ca. 50% total length. Protoconch bulbous, of 1.5 smooth, pink whorls. Teleoconch of 5 whorls, abruptly arising from protoconch. Teleoconch sculpture of ca. 17–20 flattened spiral threads,



Figures 182–195. *Diantiphos*, *Engina*, and *Hesperisternia* species. **182–185.** *Diantiphos bernardoi* (Costa and Gomes, 1998). **182.** Holotype, Museu Oceanográfico Elézer Rios da Fundação Universidade de Rio Grande, Brazil, 39.006, 15.6 mm, photo courtesy P.M. Costa (Museu Nacional, Brazil). **183–184.** GTW 9143a, 20–25 m, Guaraparí, Espírito Santo State, Brazil, 16.1 mm. **185.** HGL, 20–25 m, Guaraparí, Espírito Santo State, Brazil, 15.4 mm. **186–189.** *Diantiphos electrum* new species. **186–187.** Holotype, UF 425834, 16.7 mm. **188–189.** Paratype, BMSM 17975, from the type locality, 15.8 mm. **190–191.** *Engina goucalvesi* Coltro, 2005. GTW 12477a, 40–45 m, off Arraial do Cabo, Rio de Janeiro State, Brazil, 11.2 mm. **192–195.** *Hesperisternia itzamnai* new species. **192–193.** Holotype, UF 170226, 17.9 mm. **194–195.** Paratype, UF 170226, from the type locality, 16.2 mm.

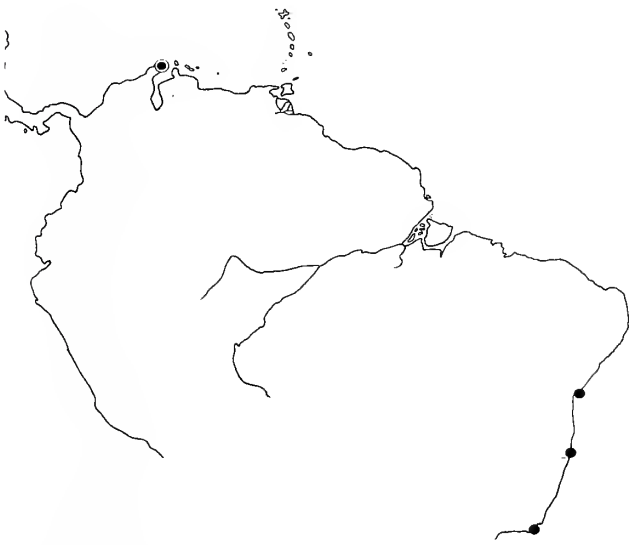


Figure 196. Distribution of *Dianthiphos bernardoi* (Costa and Gomes, 1998) (solid) and *Dianthiphos electrum* new species (bullseye).

including siphonal canal, with intercalated 2° threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of closely-spaced, low ribs; 22–28 ribs on penultimate whorl, becoming obsolete on final whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, thick. Aperture oval, outer lip without teeth. Columella angled at siphonal canal and bearing one or more weak denticles at anal canal and a single plication at siphonal canal; parietal lip adherent to previous whorl for all of its length. Siphonal canal short, open. Color yellowish-tan, darker on earliest whorls, with pale tan spiral band at periphery and faint white band anterior to that one. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: UF 425834 (ex HGL).

Type Locality: Trawled off Cabo de La Vela, Guajira Peninsula, Colombia. Depth unknown.

Paratype: BMSM 17975, 1 shell, 15.9 mm, from the type locality (ex HGL).

Distribution: Known only from the type locality.

Habitat: Based on freshly dead specimens. Depth and substrate unknown.

Etymology: Latin *electrum*, amber, in reference to the color of the shell; a neuter noun in apposition.

Discussion: See under *Dianthiphos bernardoi* for a comparison with that species. Additional specimens have recently been sold to private collectors. H.G. Lee graciously donated the specimens for study. See Table 6 for further comparison.

Genus *Monostiolum* Dall, 1904

Colubraria (*Monostiolum*) Dall, 1904: 136.
Pisania (*Monostiolum*) Dall, 1904.—Fulton, 1936: 8.
Monostiolum (*Monostiolum*) Dall, 1904.—Ponder, 1972: 255.
Caducifer (*Monostiolum*) Dall, 1904.—Cernohorsky, 1975: 196.
Monostiolum Dall, 1904.—Watters and Finlay, 1989: 48.

Type Species: By original designation, *Triton swifti* Tryon, 1881 [= *Triton tessellatus* Reeve, 1844].

Description: Small (to 21 mm), fusiform; aperture 50–70% of shell length. Protoconch of 1.25–1.5 small, smooth, tabulated whorls. Teleoconch sculpture of spiral threads and axial ribs; latter may be reduced on last ¼ whorl. Aperture with weak denticles on outer lip. Columella smooth except for denticles bounding anal and siphonal canals, angled at siphonal canal.

Discussion: Beyond the species discussed below, at least three additional ones await description. The shell illustrated in Merlano and Hegedus (1994: fig. 698) appears to represent an undescribed species but I have not seen the specimen; it is from Santa Marta, Colombia. A specimen of another undescribed species from Los Testigos, Venezuela, has been recently sold to a private collector, but the disposition of that specimen is unknown to me. A specimen of a third undescribed species from Yucatan in the García collection is too worn to be described at this time. Most of the eastern Pacific species assigned to this genus by Keen (1971) do not belong here, having different protoconchs. See Table 1 for comparison with other genera.

Monostiolum auratum Watters and Finlay, 1989
(Figures 197–201, 215)

Colubraria swifti Tyron, 1881. Warmke and Abbott, 1961: 117, pl. 21, fig. i [misidentification].
Monostiolum auratum Watters and Finlay, 1989: 51–53, figs. 3, 7E, 8; García, 2006: 80, fig. 8.

Description: Average size 18.2 mm in length (min, 15.3; max, 21.0). Fusiform; spire ca. 66% total length. Protoconch blunt, of 1.25 smooth, tabulated whorls.

Table 6. Shell characteristics of *Dianthiphos* species.

	Average length (max) mm	# spiral cords on last whorl	# axial ribs on penultimate whorl	Color
bernardoi	15.2 (19.6)	16–18	13–17	dark brown with white blotches and subperipheral band
electrum	16.3 (16.7)	17–20	22–28	yellow-tan with tan and white subperipheral bands

Teleoconch of ca. 7 whorls, abruptly arising from protoconch. Teleoconch sculpture of 20–25 rounded or flattened spiral threads, including siphonal canal, with intercalated 2° threads. Spiral cords on siphonal canal only slightly stronger. Axial sculpture of widely spaced, low ribs; 12–17 ribs on penultimate whorl. Axial ribs reduced and sigmoidal on last ½ whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, with 9–12 weak denticles on outer lip; anal and siphonal canal each set off by two denticles. Columella angled and bearing a weak denticle at siphonal canal, otherwise smooth; parietal lip adherent to previous whorl for its posterior half but erect for rest of its length. Siphonal canal short, open. Color golden orange with narrow, interrupted, white spiral bands at periphery and base. Spaces between some axial ribs dark brown; white bands do not cross these spaces. Aperture white. Operculum oval, yellow, with anterior terminal nucleus. Radula, and anatomy unknown.

Holotype: USNM 859960.

Type Locality: Rincón, Puerto Rico, in beach drift.

Paratypes: BM(NH) 1987065, 1 shell, Rincón, Puerto Rico; DMNH uncataloged, 1 shell, Rincón, Puerto Rico; DMNH uncataloged, 1 shell, beach at Piñones, 4.8 km E of Boca de Cangrejos, Puerto Rico; Finlay coll., 1 shell, Rincón, Puerto Rico; Finlay coll., 2 shells, beach at Piñones, 4.8 km E of Boca de Cangrejos, Puerto Rico; Finlay coll., 1 shell, 9–12 m, Puerto del Tortuguero, Puerto Rico.

Other Material Examined: Puerto Rico. ANSP 228472; USNM 598298, 24 km off Punta Borinquen; HGL, 1.7 m, La Parguera; GTW 8617a, 8617d, La Parguera; GTW 8617b, under rock, 13 m, Tourmaline Reef; UF 145224, Rincón; UF 388377, Playa Corcega, 2.4 km S of Rincón; UF 164005, Palmas Altas; UF 145223, San Antonio Reef; UF 145219, 162219, both Ramey Air Force Base, Aguadilla.

Distribution: Apparently endemic to Puerto Rico. Records of this species (non-types) in Watters and Finlay (1989) for St. Lucia (USNM 682388) and Barbados (USNM 19534) seem to represent aberrant *M. tessellatum* or an undescribed species.

Habitat: Fairly common in beach drift and live in rubble to 13 m.

Etymology: Latin *auratum*, golden or gilded, in reference to the color of the shell.

Discussion: In life, the shell appears dark grayish green, perhaps due to a thin periostracum, but none of the dead specimens have retained that color. The golden color and dark inter-axial streaks are characteristic of this species. See Table 7.

Monostiolum fumosum new species
(Figures 209–215)

Description: Shell 13.9–15.4 mm in length (holotype 15.4 mm in length). Fusiform; spire ca. 66% total length. Protoconch blunt, of 1.25 smooth, slightly tabulate whorls with two brown stripes. Teleoconch of 6.75 whorls, abruptly arising from protoconch. Teleoconch sculpture of rounded 1° and 2° spiral threads; 2° only evident on posterior half of whorl, of equal strength on anterior half; 20–30 threads in total including siphonal canal. Axial sculpture of widely spaced, rounded ribs; 23 ribs on last whorl, 18–24 ribs on penultimate whorl, somewhat obsolete on last ½ whorl, sigmoid in shape. Intersections of axial and spiral sculpture nodulose. Terminal varix well-developed, thick, flat, sutured, set back a short distance from outer lip. Aperture oval, with 8 thick lirae within outer lip. Columella angled and bearing a weak denticle at siphonal canal and anal canal, smooth elsewhere; parietal lip adherent for posterior third but erect for remainder of its length. Siphonal canal short, open. Color tan with brown interaxial spaces on spire, brown sutural blotches on last whorl, and a diffuse brown, subperiphreal band; the specimens examined are remarkably uniform in color and sculpture. Aperture white with columella streaked with brown. Operculum, radula, and anatomy unknown.

Holotype: UF 425833 (ex HGL).

Type Locality: 8.3 m, N side of Isla Coche, Venezuela.

Paratypes: BMSM 17978, 14.8 mm, from the type locality (ex HGL); HGL, 13.9 mm, from the type locality.

Distribution: Currently only known from the type locality.

Habitat: Based on freshly dead shells from 8.3 m. Substrate unknown.

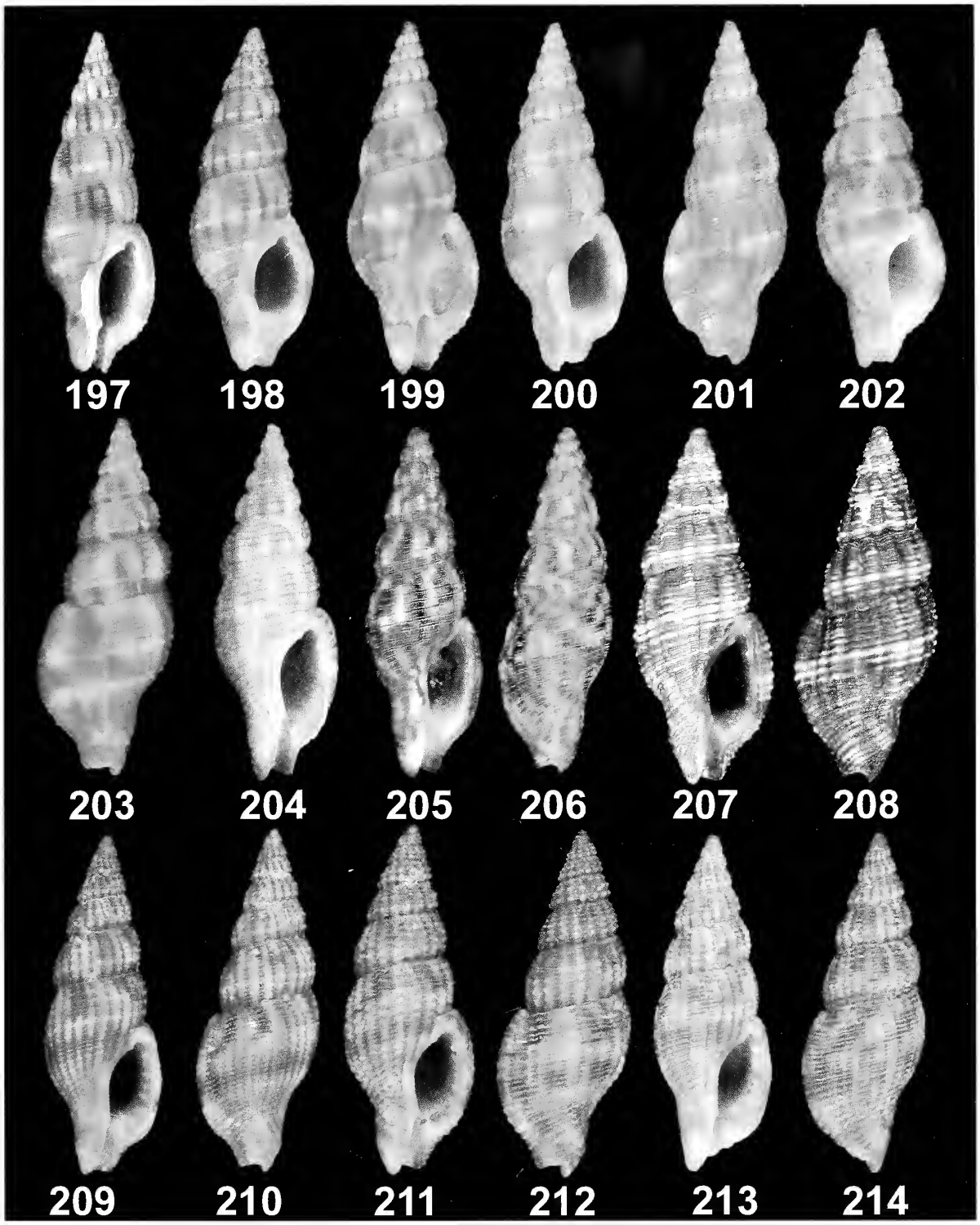
Etymology: Latin *fumosus*, smoky, in reference to the coloration of the shells.

Discussion: This species is most similar to *Monostiolum tessellatum* (Reeve, 1844). It differs in its consistently more pronounced axial sculpture that remains of almost equal strength on the last ½ whorl; in *M. tessellatum*, the axial sculpture is less pronounced overall and usually becomes obsolete on the last ½ whorl. The color pattern of *M. fumosum*, with its fine, interaxial, brown streaks, is unique among *Monostiolum*. See Table 7 for a comparison with other species. H.G. Lee graciously donated the specimens for study.

Monostiolum harryleei García, 2006
(Figures 205–206, 215)

Monostiolum harryleei García, 2006: 80–82, figs. 5, 6.

Description (Holotype): 18.9 mm in length, minus protoconch. Fusiform; spire ca. 60% total length. Protoconch unknown. Teleoconch of 6.5 whorls. Teleoconch sculpture of numerous 1°, 2°, and 3° spiral cords and threads, separated by incised lines; ca. 23 primary cords on last whorl. Axial sculpture of widely spaced, prominent ribs; ca. 15 ribs on penultimate whorl. Axial ribs



Figures 197–214. *Monostiolum* species. **197–201.** *Monostiolum auratum* Watters and Finlay, 1989. **197.** Holotype, USNM 859960, 21 mm. **198.** UF 145224, Rincón, Puerto Rico, 16.6 mm. **199.** GTW 8617c, 13 m, North Tourmaline Reef, Mayaguez, Puerto Rico, 16.4 mm. **200–201.** GTW 8617b, 13 m, Tourmaline Reef, Mayaguez, Puerto Rico, 17.5 mm. **202–204.** *Monostiolum rosewateri* Watters and Finlay, 1989. **202–203.** GTW 11416a, 83–150 m, off Baileytown, Barbados, 15.7 mm. **204.** Holotype, USNM 87098, 15.7 mm, photo courtesy of Y. Villacampa (USNM). **205–206.** *Monostiolum harryleei* García, 2007. Holotype, ANSP 413503, 18.9 mm. **207–208.** *Monostiolum nocturnum* new species. Holotype, UF 425836, 12.4 mm. **209–214.** *Monostiolum fumosum* new species. **209–210.** Holotype, UF 425833, 15.4 mm. **211–212.** Paratype, BMSM 17978, from the type locality, 14.8 mm. **213–214.** Paratype, HGL coll., from the type locality, 13.8 mm.

Table 7. Shell characteristics of *Monostiolum* species.

	Average length (max) mm	Spiral sculpture incised	# spiral cords on last whorl	# axial ribs on penultimate whorl	Axial ribs obsolete on last ¼ whorl
auratum	18.2 (21)	no	20–25	12–17	yes
fumosum	14.7 (15.4)	no	20–30	18–24	yes
harryleei	19.0	yes	23	15	yes
nocturnum	12.4	no	27	14	no
rosewateri	16.9 (18)	no	18–25	9–12	yes
tessellatum	14.9 (18)	no	25–30	15–22	yes

reduced on last 1/4 whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, with 9 weak denticles on outer lip; anal and siphonal canal each set off by two denticles. Columella angled and bearing a weak denticle at siphonal canal, otherwise smooth; parietal lip adherent to previous whorl for most of its length. Siphonal canal short, open. Color off-white with dark brown tessellations and flamulations. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: ANSP 413503.

Type Locality: 54–56 m, Bahía de Campeche, Mexico. 22°16.08′ N, 90°42.89′ W.

Paratype: EFG 25796, 1 shell, 16.7 mm, 53–55 m, Bahía de Campeche, Mexico. 22°16.45′ N, 90°39.83′ W.

Distribution: Bahía de Campeche, Mexico.

Habitat: Known only from dead shells collected at 53–56 m. Substrate unknown.

Etymology: Named for H.G. Lee, MD, of Jacksonville, Florida, renowned expert on western Atlantic mollusks.

Discussion: This species is most similar to *Monostiolum tessellatum* but differs in the peculiar incised spiral sculpture. See Table 7 for a comparison with other species.

Monostiolum nocturnum new species
(Figures 207–208, 215)

Description: 12.4 mm in length. Fusiform; spire ca. 60% total length. Protoconch blunt, of 1.25 smooth, slightly tabulate whorls; brown with two paler stripes. Teleoconch of 5.75 whorls, abruptly arising from protoconch. Teleoconch sculpture of rounded 1° and 2° spiral threads; 2° threads only evident on posterior and anterior thirds of whorl, of equal strength in middle; ca. 27 threads in total including siphonal canal. Axial sculpture of widely-spaced, rounded ribs; 13 ribs on last whorl, 14 ribs on penultimate whorl, not obsolete on last ½ whorl, sigmoid in shape on last whorl. Intersections of axial and spiral sculpture nodulose. Terminal varix well-developed, thick, flat, sutured, set back a short distance from outer lip. Aperture oval, inner surface of outer lip with large denticle at anal and siphonal canals and six much weaker, irregular denticles in between. Columella angled and bearing a weak denticle at siphonal canal and anal canal, elsewhere smooth; parietal lip adherent most of its length, barely erect on siphonal canal. Siphonal canal short, open. Color dark purplish-brown with 2–3 subperipheral spiral cords colored white; additional spiral cords forming vague, white, axial bands. Aperture purplish-brown, paler within. Operculum, radula, and anatomy unknown.

Holotype: UF 425836 (ex GTW).

Type Locality: 70–80 m, mud and sand, off Charlottesville, Tobago.

Distribution: Currently only known from the type locality.

Habitat: Based on a fresh-dead shell from 70–80 m in mud and sand.

Etymology: Latin *nocturnus*, of the night, an indirect reference to the dark-colored shell.

Discussion: Although here described from a single specimen, additional specimens from the type locality were sold to private collectors; however, the final disposition of those specimens is not known. This is a very distinct species: it is the only *Monostiolum* having the combination of prominent but closely spaced sculpture,

Figure 215. Distribution of *Monostiolum auratum* Watters and Finlay, 1989 (solid), *Monostiolum rosewateri* Watters and Finlay, 1989 (R), *Monostiolum harryleei* García, 2007 (H), *Monostiolum nocturnum* new species (bullseye), and *Monostiolum fumosum* new species (F).

dark overall coloration, and dark aperture. See Table 7 for a comparison with other species.

Monostiolum rosewateri Watters and Finlay, 1989
(Figures 202–204, 215)

Colubraria (*Monostiolum*) sp.—Sander and Lalli, 1982: 316.
Monostiolum rosewateri Watters and Finlay, 1989: 53–55, figs. 4, 8; García, 2006: 80, fig. 10.

Description: Average size 16.9 mm in length (min, 15.8; max, 18.0). Fusiform; spire ca. 60% total length. Protoconch blunt, of 1.5 smooth, tabulated whorls. Teleoconch of ca. 7 whorls, abruptly arising from protoconch. Teleoconch sculpture of 18–25 rounded or flattened spiral threads, including siphonal canal, with intercalated 2°threads. Spiral cords on siphonal canal only slightly stronger. Axial sculpture of widely spaced, prominent ribs, 9–12 ribs on last whorl. Axial ribs reduced in strength on last ½ whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, thick, set back a short distance from outer lip. Aperture oval, with 7–9 lirate teeth on outer lip; anal and siphonal canal each set off by two denticles. Columella angled and bearing a weak denticle at siphonal canal, elsewhere smooth; parietal lip adherent to previous whorl for most of its length. Siphonal canal short, open. Color cream to tan with irregular white blotches and two vague, white, spiral bands at periphery and base. In some specimens primary spiral cords are brown, but other shells do not show this feature. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: USNM 87098.

Type Locality: Western Barbados, BLAKE Sta. 272, 139 m, ca. 13°10′ N, 59°40′ W.

Paratypes: AMNH 112353, 2 shells, W side Barbados; Redpath Museum 16301, 1 shell, DIADEMA Sta. 55, 229 m, off St. James and Speightstown, western Barbados, on sandy bottom.

Other Material Examined: Barbados. Redpath Museum, uncataloged, DIADEMA Sta. 69, 186 m, off Coral Beach, sand and shell bottom; GTW 11416a, 83–150 m, off Baileytown; HGL, W Holetown, St. James.

Distrubution: Endemic to the SW coast of Barbados.

Habitat: Dead shells are found on sand and shell bottoms at 139–229 m.

Etymology: Originally named after the late Joseph Rosewater of USNM in recognition of his many malacological achievements and his kindness to the author during my visits there.

Discussion: This species is apparently endemic to fairly deep water off western Barbados. It is easily differentiated from *Monostiolum tessellatum*, which occurs in much shallower water in Barbados, by the more pronounced and fewer axial ribs (ca. 9–12 in *M. rosewateri* vs. ca. 15–22 in *M. tessellatum*). See Table 7 for a comparison with other species.

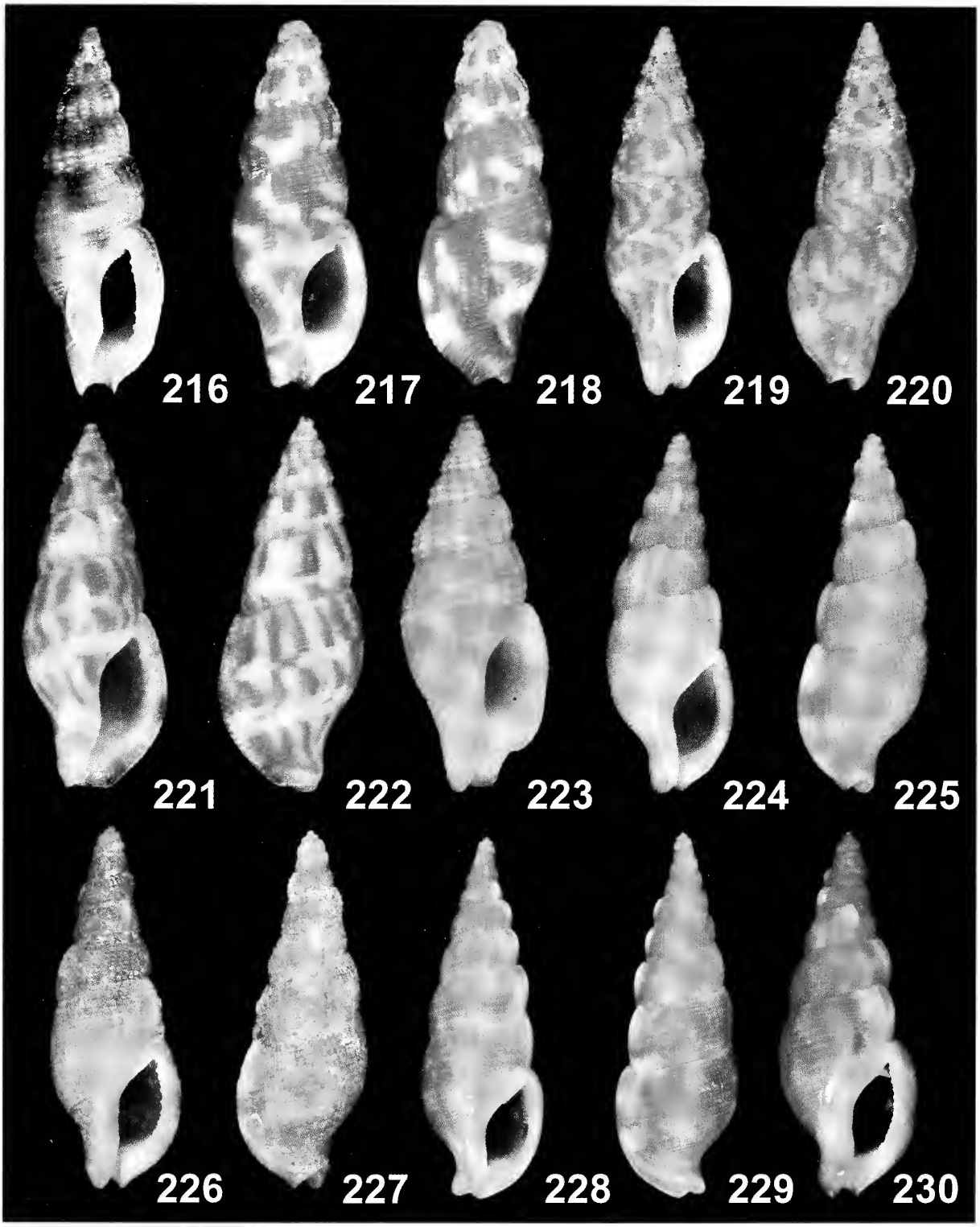
Monostiolum tessellatum (Reeve, 1844)
(Figures 216–223, 231)

Triton tessellatus Reeve, 1844: pl. 19, fig. 91; Tryon, 1881: 30 [in synonymy of *Triton concinnus* Reeve, 1846].
Pleurotoma igniflua Reeve, 1845: pl. 24, fig. 214.
Triton (*Epidromus*) *swifti* Tryon, 1881: 31, pl. 16, fig. 158.
Triton swifti Tryon, 1881.—Simpson, 1887: 65.
Colubraria swiftii [*sic*] (Tryon, 1881).—Dall, 1889a: 19, 226 [in part].
Colubraria (*Monostiolum*) *swifti* (Tryon, 1881).— Dall, 1904: 136.
Pisania (*Monostiolum*) *igniflua* (Reeve, 1845).—Fulton, 1936: 7, 8.
Monostiolum (*Monostiolum*) *swifti* (Tryon, 1881).—Ponder, 1972: 255, pl. 24, fig. 7, text fig. 1.8.
Caducifer (*Monostiolum*) *tessellatus* (Reeve, 1844).—Cernohorsky, 1975: 196, fig. 50.
Caducifer (*Monostiolum*) *swifti* (Tryon, 1881).—Watters, 1983: 125, 126, figs. 7–10, 12.
Monostiolum tessellatum (Reeve, 1844).—Beu and Maxwell, 1987: 59; García, 2006: 80, fig. 9.
Monostiolum swifti (Tryon, 1881).—Beu and Maxwell, 1987: 59.

Description: Average size 14.9 mm in length (min, 12.3; max, 18.0). Fusiform; spire ca. 50–66% total length. Protoconch blunt, of 1.5 smooth, tabulated whorls. Teleoconch of ca. 7 whorls, abruptly arising from protoconch. Teleoconch sculpture of 25–30 rounded or flattened spiral threads, including siphonal canal, with intercalated 2° threads; these 2° threads may become equal in strength to 1° ones on last whorl. Spiral cords on siphonal canal stronger and flattened. Axial sculpture of widely spaced, low ribs, 15–22 ribs on last whorl. Axial ribs reduced or barely perceptible on last ½ whorl. Intersections of axial and spiral sculpture weakly nodulose. Terminal varix well-developed, set back a short distance from outer lip. Aperture oval, with ca. 9 weak denticles on outer lip; anal and siphonal canal each set off by two denticles. Columella angled and bearing a weak denticle at siphonal canal, elsewhere smooth; parietal lip adherent to previous whorl for its posterior half but erect for rest of its length. Siphonal canal short, open. Color pattern quite variable, ranging from nearly all white to all dark brown, usually with zig-zag flammulations or checkerboard pattern. A vague basal band of white may be present as well. Operculum rhomboidal, tan, with anterior terminal nucleus. Radula figured by Ponder (1972: fig. 1.8); central tooth with three cusps; laterals with three cusps, outer cusp largest. Anatomy unknown.

Types: *Triton tessellatus* Reeve, 1844; lectotype by designation of Watters and Finlay (1989), BM(NH) 196747/1. *Pleurotoma igniflua* Reeve, 1845, type(s) apparently lost. *Triton* (*Epidromus*) *swifti* Tryon, 1881, holotype ANSP 59208.

Type Locality: *Triton tessellatus* Reeve, 1844, "Island of Burias, Philippines" corrected by Watters and Finlay (1989) to Barbados. *Pleurotoma igniflua* Reeve,



Figures 216–230. *Monostiolum* and *Cunuia* species. **216–223.** *Monostiolum tessellatum* (Reeve, 1845). **216.** Lectotype of *Triton tessellatus* Reeve, 1844, BM(NH) 196747/1, photo from Watters and Finlay (1989), 16.6 mm. **217–218.** HGL, The Reefs, Southhampton, Bermuda, 15.4 mm. **219–220.** GTW 4068a, Bermuda, 17.5 mm. **221–222.** GTW 4068b, Tiburon, Haiti, 12.6 mm. **223.** GTW 8617c, 5 m, E side of Booby Point, Tobago, 12.1 mm. **224–227.** *Cunuia clavula* new species. **224–225.** Holotype, UF 341080, 18.1 mm. **226–227.** Paratype, BMSM 17973, Palenque, Dominican Republic, 13.6 mm. **228–230.** *Cunuia suuderlaudi* (Petuch, 1995). **228–229.** Holotype, UF 225165, 20 mm. **230.** HGL, 27 m, Tryall, Jamaica, 18.2 mm.

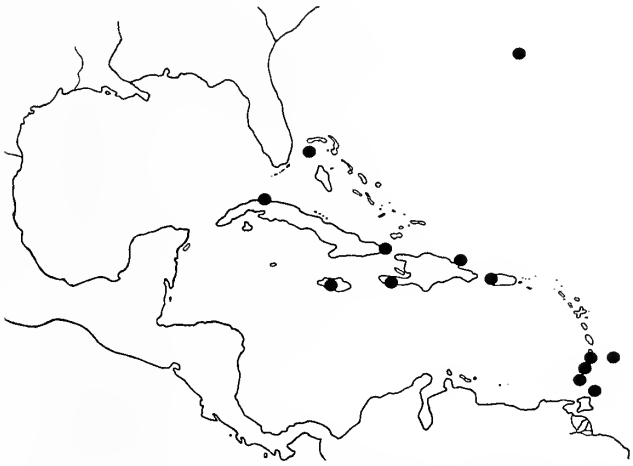


Figure 231. Distribution of *Monostiolum tessellatum* (Reeve, 1845).

1845, unknown. *Triton* (*Epidromus*) *swifti* Tryon, 1881, Antigua.

Paratypes: *Triton tessellatus* Reeve, 1844, 3 paralectotypes by designation of Watters and Finlay (1989), BM (NH) 196747/2–4.

Other Material Examined: Bermuda. ANSP 10145, 17822, 36217, 36326, 70156, BMSM 38496, UF 56460, 70372, 154832, 214436, 390474, DMNH 24501, USNM 94410, 149864, 221621, 417730, 663420, GTW 4068a; USNM 656480, NW reefs off Somerset; USNM 658971, SW reef off Somerset; UF 145214, 145215, 145222, both Hastings Rocks, Bridgetown; UF 145221, 145226, both Hungry Bay, S shore; ANSP 319019, Hungry Bay; USNM 714206, Tucker's Town; USNM 771849, Castle Harbour, Blue Hole; USNM 807649, St. George's Island; USNM 621601, W end of St. George's Island; HGL, intertidal, Southampton, The Reefs; DMNH 51840, Bailey's Bay; ANSP 145957, Shelly Bay; ANSP 88579, USNM 171930, both Gibbet Island; ANSP 183806, USNM 152157, both Hamilton; USNM 835691, SW of Whalebone Bay; DMNH, Coney Island, off Ferry Reach; AMNH 193322, USNM 500148. Bahamas. USNM 54542; USNM 417731, Bimini. Cuba. UF 145225, Las Carboneras, Varadero, Matanzas Province; USNM 678505, Guantánamo Bay, Guantánamo Province. Jamaica. ANSP 36219, 36220. Haiti. BMSM 38497, Tiburon; GTW 4068b, under rubble on reef, shallow water, Tiburon. Dominican Republic. USNM 42964, Samaná. Puerto Rico. UF 162220, Rincón. Bequia. HGL, 3.3 m. Grenada. Finlay coll. Barbados. UF 145220; USNM 500149, 22 m, Carlisle Bay; USNM 500150, 4.6–6 m, off Pelican Island; USNM 459598, shallow water, off Pelican Island. Trinidad and Tobago. AMNH 193453, USNM 682304, both shallow water, Buccoo reef, Tobago; UF 145218, 12 m, Buccoo Point, Tobago; UF 145217, Buccoo Point, Tobago; Finlay coll., Arnos Vale beach, Tobago; HGL, Monkey Point, E coast, Tobago; GTW 8617c, 5 m, E side of Booby Point, Mt. Irving Bay, Tobago.

Distribution: Islands in the western Atlantic Ocean: Bermuda, Greater and Lesser Antilles; possibly St. Lucia (see under *M. auratum*). The Brazilian record for this species in Watters and Finlay (1989), based on a single juvenile individual (Rios, 1994: pl. 39, fig. 514 and in subsequent editions), is now interpreted as a juvenile of *Caducifer atlanticus* Coelho, Matthews and Cardoso, 1970.

Habitat: Dead shells are found from shallow water to at least 33 m under rubble on reefs. Live-taken specimens are rare.

Etymology: Latin *tessellatus*, mosaic. The specimen described by Reeve had a checkerboard pattern.

Discussion: In contrast to the other species of Atlantic *Monostiolum*, *M. tessellatum* has a very wide distribution; the remaining species are all narrowly endemic. Nevertheless, *M. tessellatum* appears to be rare outside of Bermuda and Barbados, the two extremes of its range. The name "*Colubraria swifti*" has stubbornly persisted despite the fact that the valid name for this species is *Monostiolum tessellatum*. The taxonomic tangle of *swifti*/*tessellatum* was described in detail in Watters and Finlay (1989). See Table 7 for a comparison with other species.

Genus *Parviphos* Sarasua, 1984

Parviphos Sarasua, 1984: 2.

Type Species: *Phos adelus* Schwengel, 1942, by original designation (see discussion).

Description: Small (to 16 mm), compact, solid shells. Protoconch of 1.5 smooth whorls, tabulate, with first whorl sunken into the remainder. Spire usually ca. 50% of overall height. Sculptured with axial ribs and spiral threads. No previous varices. Final varix massive, reflected abaperturally. Columella with or without denticles. Inner surface of outer lip with strong lirae. Anal canal bounded by two prominent denticles. Juveniles of *P. chalcedonius* new species have a thin periostracum bearing minute bristles; this has not yet been observed on other species.

Discussion: In the UF collection are specimens of this genus listed under the name "*Spartaphos*;" this is a manuscript name attributed to H. Rehder but never validly introduced. Sarasua (1984) originally compared this genus to *Antillophos*, noting the lack of a transition between the protoconch and the teleoconch in *Antillophos* that is more apparent in *Parviphos*. The protoconchs of the two genera actually bear no resemblance to each other. In *Parviphos* the protoconch is smooth, small, tabulate, with the first whorl sunken into the next; in *Antillophos* the protoconch is larger and conical with a sharp peripheral keel. The protoconch of *Parviphos* is more similar to that found in *Monostiolum*.

Parviphos differs from *Engina* in having lirae rather than denticles within the outer lip, a massive, reflected terminal varix, and none or reduced columellar

denticles. *Pollia* Gray, 1834, type species *P. undosum* (Linnaeus, 1758), differs in having a labral tooth on the outer lip. Many species need to be reexamined in light of these differences. For instance, the syntype of "*Pollia*" *eximia* (Reeve, 1846) illustrated by Kaicher (1990: No. 5839), appears congeneric with *Parviphos*. See Table 1 for comparison with other genera.

Sarasua (1984) gave *Phos adelus* as the type of the genus. However, she did not illustrate an example and in Sarasua and Espinosa (1984) a specimen of *P. chalcedonius* n.sp. is illustrated as "*Phos adelus*." This suggests that the type species may have been misidentified, but lacking the specimen(s) upon which the genus was established I cannot be sure. Nevertheless, both *P. adelus* and *P. chalcedonius* are congeneric.

Parviphos adelus (Schwengel, 1942)
(Figures 232–244, 247)

Phos (?) *adelus* Schwengel, 1942: pl. 3, fig. 4 [July], 66 [Oct.]; the captioned plate was published prior to the text description.
Antillophos adelus (Schwengel, 1942).—Kaicher, 1986: No. 4442.
Parviphos adelus (Schwengel, 1942).—Sarasua, 1984: 2; Watters, 2007: 10.

Description: Average size 14.1 mm in length (min, 12.9; max, 16.2). Biconical, rather wide; spire ca. 50% total length. Protoconch small, flattened, of 1.5 smooth, tan to purple whorls, with a paler band. Teleoconch of 5–5.75 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 17–18 rounded, erect, widely separated spiral cords, including siphonal canal, with intercalated 2° threads or cords, and occasionally 3° threads. 2° therads may be as large as 1° cords in some specimens. Axial sculpture of widely spaced, high ribs; 10–13 ribs on penultimate whorl, 9–11 ribs on last whorl, not including varix. Intersections of axial and spiral sculptured with strong, elongated nodules. Terminal varix well-developed, reflected, somewhat constricted, thick, wide, with 1–3 axial swellings; often preceded by a wide, flat space on whorl. Aperture oval, outer lip with 10–14 sharp, lirate teeth; canals bounded by larger teeth. Columella angled at siphonal canal, bounded by two plications; one denticle bounding anal canal on columella. Columella with 4–11 weak denticles. Siphonal canal short, open. Color yellowish tan with interspaces of spiral threads colored brown as they pass over axial ribs; wide, white peripheral band is always evident. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: ANSP 178477, lost (*fide* P. Callomon, pers. comm., 2008) but previously illustrated in Kaicher (1986: No. 4442), reproduced here.

Type Locality: Puerto Plata, Dominican Republic.

Other Material Examined: Costa Rica. UF 383284, 388334, both Moín Bay. Bahamas. GTW 6735b, 13.3 m, Start Bay, Mayaguana Island. Puerto Rico. HGL, UF 158056, both Piñones Beach, San Juan; UF 163093, N Mayaguez. Cuba. UF 55712, Guantánamo, Guantánamo Province. Barbados. UF 266957. Colombia. GTW 6735f, 8 m, Cabo de La Vela, La Guajira Peninsula.

Distribution: The range of this very rare species has not been adequately delineated. It has been recorded from the central Antilles, Costa Rica, Barbados, and Colombia.

Habitat: Dead shells have been recorded from 8 m in a sand substrate.

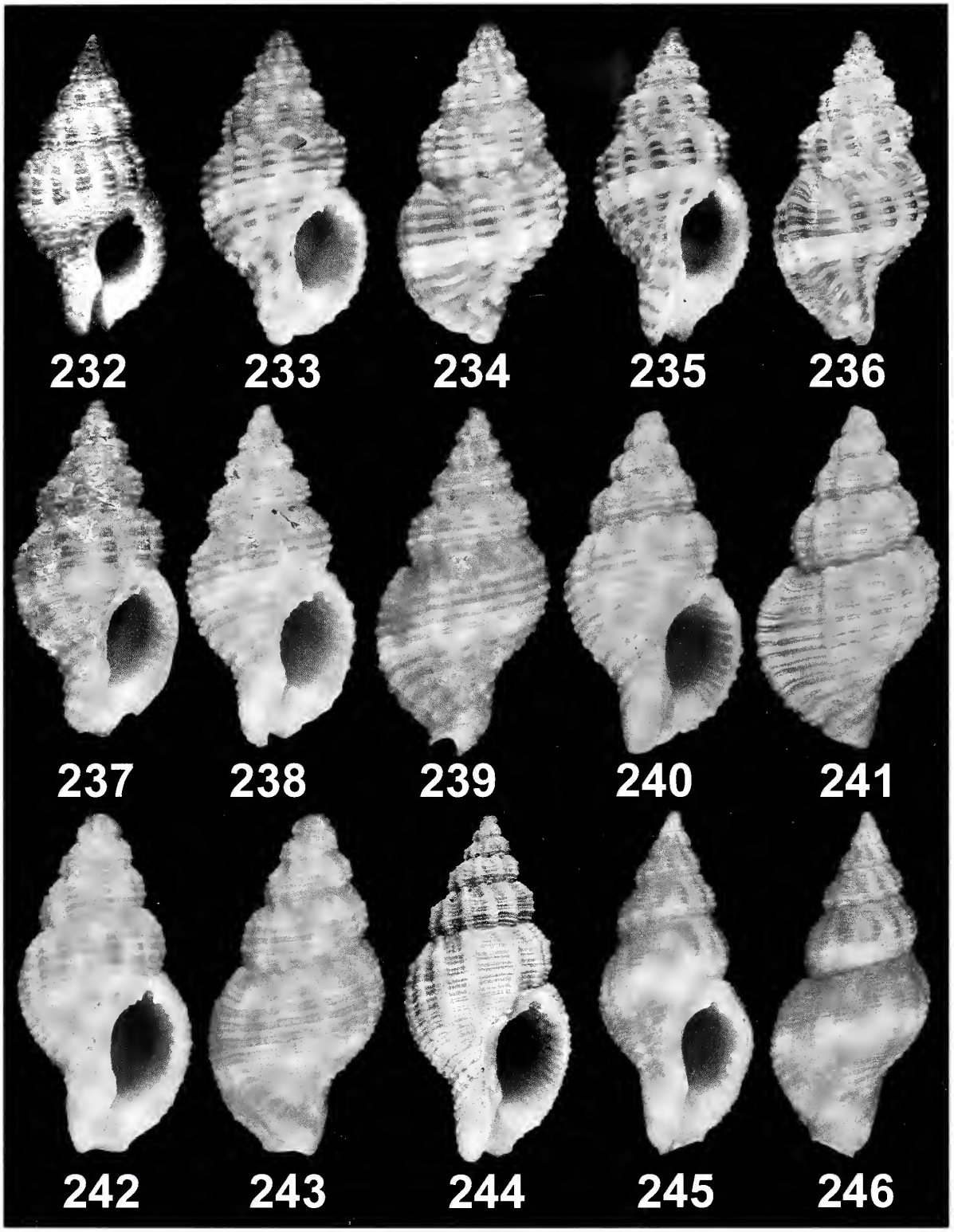
Etymology: Greek *adelos*, unknown, obscure, in reference to the long hidden nature of this species.

Discussion: The holotype is lost but the original figure and Kaicher (1986) clearly depicts the species discussed here. Most records of this species are for the similar *P. chalcedonius* new species. It differs from *P. chalcedonius* in generally having fewer axial ribs (10–13 on the penultimate whorl in *P. adelus* vs. 12–17 in *P. chalcedonius*), which are more prominent and separated by deeper interspaces in *P. adelus*. *Parviphos chalcedonius* also has more lirae on the inner side of the outer lip (14–19) than does *P. adelus* (10–14). The color pattern of *P. adelus* is very uniform: darker axial ribs with a prominent peripheral white band; *P. chalcedonius* has a color pattern of brown splotches and dots with a white band (rarely absent). Some individuals of *Anna milleri* are similarly colored but that species is much smaller and lacks the reflected terminal varix. The eastern Pacific Ocean *P. nigricostatus* (Reeve, 1846) is the cognate of *P. adelus*; it somewhat larger and darker in color but otherwise has the same overall sculpture and color pattern. This is the first recognition of *Parviphos* in the Pacific Ocean. See Table 8 for a comparison with other species.

Parviphos chalcedonius new species
(Figures 248–263)

Antillophos oxyglyptus Dall and Simpson, 1901. Warmke and Abbott, 1961: 115, pl. 21, fig. g [misidentification].
Bailya parva (Adams, 1850).—Humphrey, 1975: pl. 17, figs. 21, 21a [misidentication].
Phos adelus Schwengel, 1942.—Sarasua and Espinosa, 1984: 7, fig. 4c [misidentification]; Robin, 2008: 183, fig. 3 [misidentification].
Parviphos adelus (Schwengel, 1942).—Redfern, 2001: 92, pl. 43, fig. 391 [misidentification].

Description: Shell 11.6–16.7 mm in length (holotype 13.9 mm in length). Fusiform; spire 50 – 60% total length. Protoconch small, of 1.5 smooth, white whorls with tan blotches. Teleoconch of 5 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 27 rounded, widely-separated, spiral threads, including siphonal canal, with intercalated microscopic threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely spaced, high ribs; 12–17 ribs on penultimate whorl, 18 ribs on last whorl, becoming obsolete on last ½ whorl, not including varix. Intersections of axial and spiral sculptured with strong,



Figures 232–246. *Parviphos* species. **232–244.** *Parviphos adelus* (Schwengel, 1942). **232.** Holotype, ANSP 178477, reproduced from Kaicher (1946), No. 4442, 16.5 mm. **233–234.** UF 158056, Piñones Beach, San Juan, Puerto Rico, 15.3 mm; **235–236.** GTW 6735b, 13.3 m, Start Bay, Mayaguana Island, Bahamas, 14.4 mm; **237.** UF 266958, Punta Galeta, Isla Galeta, Panama, 14.4 mm. **238–239.** UF 55712, Guantánamo, Guantánamo Province, Cuba, 13.1 mm; **240–241.** UF 266957, Barbados, 14.3 mm; **242–244.** UF 383284, Moín Bay, Costa Rica, **242–243.** 13.9 mm; **244.** 16.2 mm (bleached). **245–246.** *Parviphos marijkae* (De Jong and Coomans, 1988). Holotype, ZMA 3.87.082, 16.7 mm.

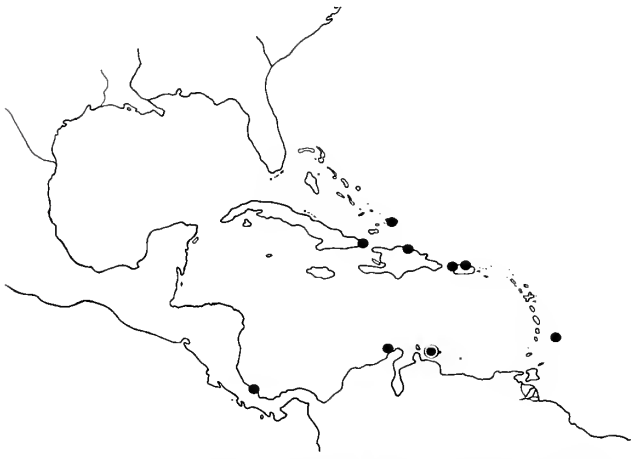


Figure 247. Distribution of *Parviphos adelus* (Schwengel, 1942) (solid) and *Parviphos marijkae* (De Jong and Coomans, 1988) (bullseye).

elongated nodules. Terminal varix well-developed, reflected, somewhat constricted, wide, thick. Aperture oval, inside of outer lip with 14–19 lirae. Columella angled at siphonal canal; anal canal bounded by a denticle, siphonal canal bounded by weak lirae, remainder of columella with 4–10 weak denticles; parietal lip barely adherent. Siphonal canal short, open. Color white with brown patches, often more or less aligned with axial ribs, and wide subperipheral white band; the intensity of the color varies considerably but the pattern is fairly uniform. The white subperipheral band is rarely absent. Aperture white. Operculum, radula, and anatomy unknown. Juveniles have a thin periostracum with minute bristles.

Holotype: UF 425829.

Type Locality: 30 m, Mariel sands, La Habana Province, Cuba.

Paratypes: UF 425830, 1 shell, 15.3 mm, from type locality; UF 150208, 3 shells, 12.5, 13.5, 13.8 mm, Hog Island, off New Providence, Bahamas; BMSM 17980, 1 shell, 13.8 mm, 5 m, at night, Honeymoon Cove, Gun Cay, Bahamas (ex GTW).

Other Material Examined: Mexico. UF 361554, Cayos Arcas; EFG 26051, 52–53 m, 22°16′ N, 90°43′ W, off Mérida. Belize. EFG 10609, off Cay Bokei, Turneffe Islands. Honduras. EFG 9221, 10 m, Lagoon Reef, Utila Island; HGL, Caribe Bight, Roatán Island; EFG 5383, 0.6 m, Caribe Point, Roatán Island. Panama. UF 266958, Punta Galeta, Isla Galeta. Florida. UF 352845, Delray Beach, Palm Beach Co.; UF 157577, 20 m, Pompano Beach fill, Broward Co.; UF 120740, 6.7 m, Key Largo, off Pickles Reef, Monroe Co.; FMNH 315221, 2–5 m, Carysfort Reef, off Key Largo, Monroe Co., 25°13′ N, 80°12′ W; FMNH 150205, Carysfort Reef, off Key Largo, Monroe Co., FMNH 315163, 7 m, Molasses Reef, Key Largo, Monroe Co.; GTW 6735a, under rubble, 3.3–6.6 m, Fowey Rocks, Key Largo, Monroe Co. Florida; BMSM 8003, Sombrero Key, Monroe Co.; FMNH 289069, 8 m, Looe Key, Big Pine Key, Monroe Co.; UF 121777, Looe Key, Big Pine Key, Monroe Co.; FMNH 154783, Fort Jefferson, Dry Tortugas; UF 425831, Dry Tortugas. Bahamas. BMSM 38498, 2.6–3.3 m, Tarpum Bay, Eleuthera; UF 267201, Nassau, New Providence Island; UF 352844, 26.7–28.3 m, Gold Rock, S shore Grand Bahama Island; UF 150207, 150210, both Rose Island; CR 8571, 10 m, Chub Rocks, Abaco, 26°44′ N, 77°13′ W. Cuba. UF 150209, 30 m, Mariel sands, La Habana Province; UF 266959, 397260, both Varadero, Matanzas Province. Puerto Rico. UF 164191, La Parguera; UF 164328, 5 m, Icacos. US Virgin islands. UF 154782, 266957, 397108, all Water Island. Antigua. GTW 6735d, 6–10 m, coral rubble, Falmouth. Trinidad and Tobago. UF 281381, Scarborough. Colombia. GTW 6735e, on rubble bottom, 2–4 m, Islas de Rosario, Cartagena.

Distribution: Widely distributed from the eastern Gulf of Mexico throughout the Caribbean Sea to Colombia and Tobago.

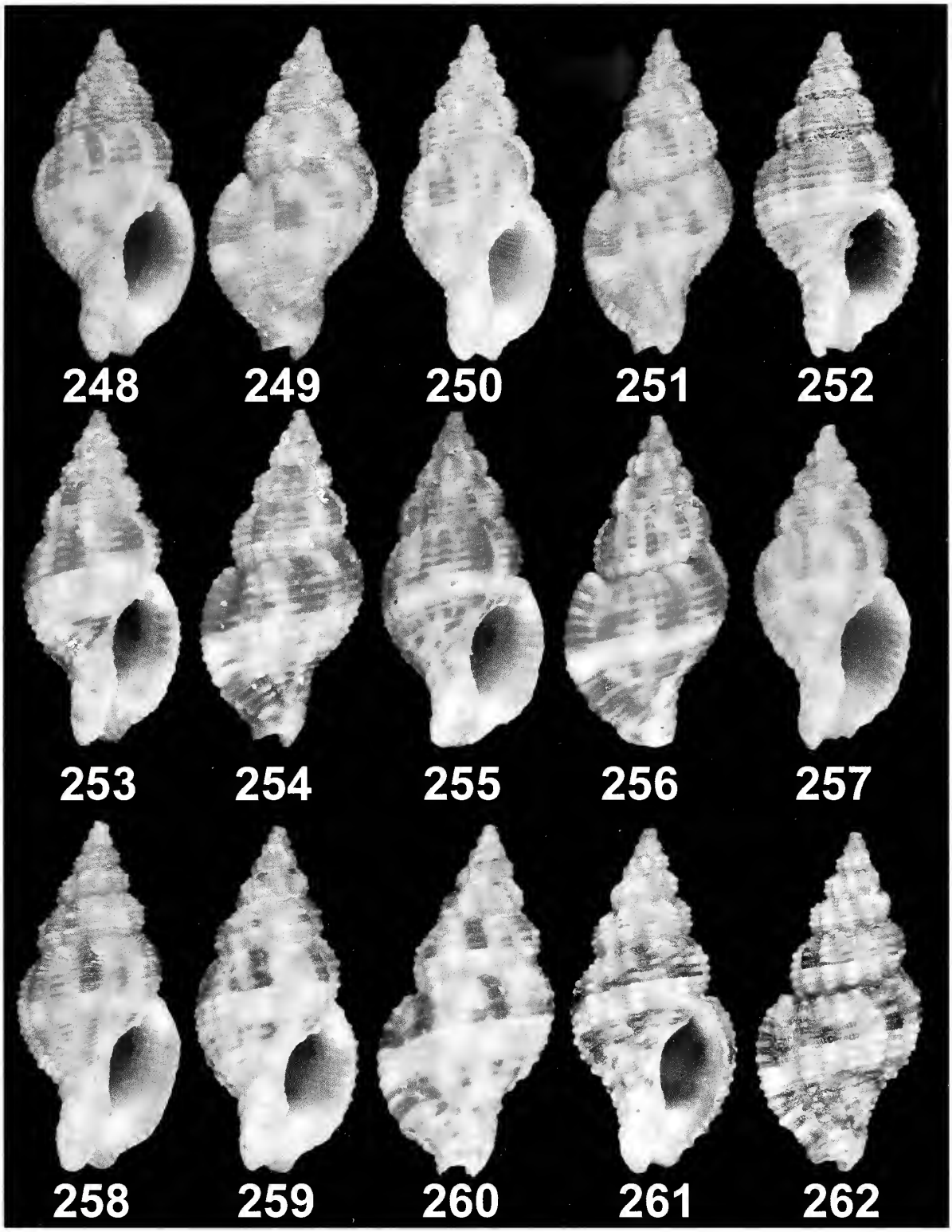
Habitat: Dead shells have been recorded from 0.6–30 m. Live specimens are associated with coral rubble and under rocks at 5–30 m.

Etymology: Latin *chalcedonius*, resembling the mineral chalcedony.

Discussion: This species has been confused in the literature and in collections with *Parviphos adelus*, which appears to be a much rarer species and to have a more limited distribution, and with *Bailya parva*, to which its bears no resemblance. It is not particularly rare, only misunderstood. *Parviphos chalcedonius* has more axial ribs and more inner lip lirae than in *P. adelus*. The color patterns are also quite different. See the

Table 8. Shell characteristics of *Parviphos* species.

	Average length (max) mm	# axial ribs on penultimate whorl	# denticles on columella	# lirae on inner surface of outer lip	color
adelus	14.1 (16.2)	10–13	4–11	10–14	White with dark axials and white band
chalcedonius	13.6 (16.7)	12–17	4–10	14–19	White with brown patches and white band
marijkae	16	14	7	9	Orange-tan with white band



Figures 248–262. *Parviphos chalcedonius* new species. **248–249.** Holotype, UF 425829, 13.9 mm. **250–251.** Paratype, UF 425830, from type locality, 15.3. **252.** UF 281381, Scarborough, Tobago, 13.9 mm. **253–254.** Paratype, UF 150208, Hog Island, off New Providence, Bahamas, 13.8 mm. **255–256.** UF 120740, 6.7 m, Key Largo, off Pickles Reef, Monroe Co., Florida. **257.** UF 266959, Varadero, Matanzas Province, Cuba, 13.5 mm. **258.** GTW 6735e, 2–4 m, Islas de Rosario, Cartagena, Colombia, 13.6 mm. **259–260.** GTW 6735a, 3.3–6.6 m, Fowey Rocks, Key Largo, Monroe Co., Florida, 13.1 mm. **261–262.** GTW 6735d, 6–10 m, Falmouth, Antigua, 13.7 mm.

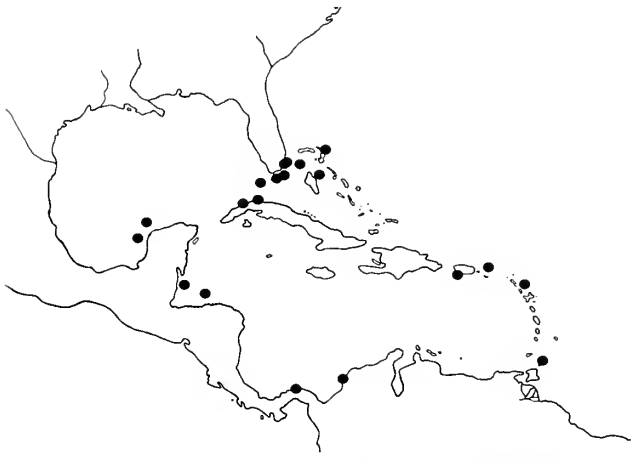


Figure 263. Distribution of *Parviphos chalcedonius* new species.

discussions under *P. adelus* and *P. marijkae* for additional comparisons with those species and Table 8.

Parviphos marijkae (De Jong and Coomans, 1988)
(Figures 245–247)

Bailya marijkae De Jong and Coomans, 1988: 82, pl. 38, fig. 449; Faber, 2007: 74, figs. 11, 12 [in synonymy of *P. milleri*].
"*Bailya*" *marijkae* De Jong and Coomans, 1988.—Watters, 2007: 10.

Description: 16.7 mm in length. Fusiform; spire ca. 60% total length. Protoconch small, of 1.5 smooth, somewhat flattened, white whorls. Teleoconch of 5 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 28 rounded, widely separated, narrow, spiral cords, including siphonal canal, with intercalated microscopic threads. Axial sculpture of widely spaced, low ribs; ca. 14 ribs on penultimate whorl, ca. 13 ribs on last whorl, becoming obsolete and sigmoidal on last ½ whorl, not including varix. Intersections of axial and spiral sculptured with weak, elongated nodules. Terminal varix well-developed but low, not reflected. Columella with 7 wide denticles, canal bounded by denticle, siphonal canal bounded by weak lirae; parietal lip thick, erect on anterior 3/4ths. Inner surface of outer lip with 9 lirae, largest at the anal canal. Siphonal canal short, open. Color orangish-tan, darkest on axial ribs, with wide, white peripheral band. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: ZMA 3.87.082.

Type Locality: Curaçao.

Distribution: Known only from Curaçao.

Habitat: Unknown.

Etymology: Named after Marijke de Jong, daughter of K.M. de Jong.

Discussion: The holotype was the only specimen of *P. marijkae* available for study, and no paratypes were mentioned, although the original description stated that other specimens were known to the authors from Awa di Oostpunt, Schottegat, "and other localities in Curaçao." Faber (2007) synonymized *B. marijkae* with *A. milleri*; however, its closest relatives are *P. chalcedonius* and *P. adelus*. It differs from *A. milleri* in its greater size (16 vs. 11 mm), more elongate shape, greater number of axial ribs on the penultimate whorl (14 vs. 9), and obsolete axials on the last ½ whorl. It differs from *P. chalcedonius* and *P. adelus* in having fewer lirae on the inner lip, fewer columellar denticles, a less massive, nonreflected terminal varix, and a different color pattern. See Table 8 for a comparison with other species.

Genus *Engina* Gray, 1839

Engina Gray, 1839: 112–113.

Type Species: *Engina zonata* Gray, 1839, by subsequent designation of Gray (1847) [= *Purpura turbinella* Kiener, 1835, see Orr (1962)].

Discussion: The genus *Engina*, based on *E. turbinella* (Kiener, 1835), encompasses a wide variety of conchologically disparate species requiring reallocation that is beyond the immediate scope of this study. The type "species" itself probably contains several different species. One species, *Engina goncalvesi* Coltro, 2005, is discussed here because of its affinities to, and reported synonymy with, *P. milleri*.

Engina goncalvesi Coltro, 2005
(Figures 190, 191)

Engina goncalvesi Coltro, 2005: 1–2, pl. B, figs. 1–11; Faber, 2007: 74 [in synonymy of *Engina milleri* (Usticke, 1959)].

Description: Average size 12.1 mm in length (min, 11.0; max, 14.2). Fusiform; spire ca. 50% . total length. Protoconch small, of 1.5 smooth, brown whorls with pale peripheral band. Teleoconch of 5 whorls, abruptly arising from protoconch. Teleoconch sculpture of ca. 20 flattened, spiral threads, including siphonal canal, with numerous intercalated 2° and 3° threads. Spiral cords on siphonal canal slightly stronger. Axial sculpture of broad, low ribs; ca. 20 ribs on penultimate whorl, obsolete on most specimens by last whorl. Intersections of axial and spiral sculptured with weak, elongated nodules. Terminal varix well-developed, flaring, moderately narrow. Aperture oval, outer lip with 6–7 teeth. Columella angled at siphonal canal and bearing ca. 5 irregular denticles on anterior half, a single lirate denticle bounding anal canal; parietal lip erect for most of its length. Siphonal canal short, open. Color brown with pale tan spiral band at sub-periphery, primary spirals often darker. Aperture with brownish-purple tinge. Operculum leaf-shaped, golden-tan, with anterior terminal nucleus. Radula and anatomy unknown.

Holotype: Stated to be in Museu de Zoologia da Universidade de São Paulo, MZSP 37179, but not found (*fide* L. R. L. Simone, pers. comm., 2008).

Type Locality: Off Cabo Frio, Rio de Janeiro State, Brazil.

Paratypes: Museu Oceanográfico Eliézer Rios da Fundação Universidade de Rio Grande, Brazil, MORG 43854, 1 shell; Museu Nacional da Universidade Federal do Rio de Janeiro, Brazil, unnumbered, 2 shells; P.M. Santos Costa coll., 1 shell. The localities of the paratypes were not given and are presumed to be from the type locality.

Other Material Examined: Brazil. GTW 12477a, on rocks in caves at 40–50 m, off Arraial do Cabo, Rio de Janeiro State.

Distribution and Habitat: "Lives under rocks at 25–35 meters, between Cabo Frio, Rio de Janeiro State and Ilhabela, São Paulo State" (Coltro, 2005: 2). Additional records here increase the depth of live-taken individuals to 45 m.

Etymology: Named for Paulo Cesar Pinto Gonçalves, discoverer of the species.

Discussion: This species is somewhat similar to *A. milleri* (Utsicke, 1959) and was synonymized with it by Faber (2007). I feel it is distinct. *Engina goncalvesi* differs from *A. milleri* in the following ways: in *E. goncalvesi* the axial sculpture is obsolete on the last whorl but remains rather prominent in *A. milleri*; the terminal varix in *E. goncalvesi* is flared and relatively narrow (a characteristic of *Engina*), in *A. milleri* it is somewhat constricted and much thicker (characteristic of *Anna*); the siphonal canal is longer and straighter in *E. goncalvesi* than in *A. milleri*; in *E. goncalvesi* there are ca. 13 axial ribs on the penultimate whorl in contrast to 8–9 ribs in *A. milleri*; *E. goncalvesi* lacks denticles on the posterior half of the columella except for a single lirate tooth bordering the anal canal whereas *A. milleri* has a series of distinct denticles along the entire length of the parietal lip. *Engina demani* De Jong and Coomans, 1988, from the Netherlands Antilles is very similar but has stronger sculpture that persists on the final whorl; the aperture of *E. goncalvesi* is pale purple and brown whereas the aperture of *E. demani* is white.

Hesperisternia Gardner, 1944

Type species: *Hesperisternia waltoni* Gardner, 1944, by original designation.

Hesperisternia itzamnai new species
(Figures 192–195, 264)

Description: Shell 16.2 (broken)–17.8 mm in length (holotype 17.8 mm in length). Fusiform; spire ca. 50% total length. Protoconch small, conical, of 1.5 smooth, white whorls with tan blotches. Teleoconch of 5.5 whorls, strongly demarcated from protoconch. Teleoconch sculpture of ca. 13 rounded, widely-separated spiral threads, including siphonal canal, with numerous intercalated 2° threads. Subsutural area wide, flat, with single primary thread. Spiral cords on siphonal canal slightly stronger. Axial sculpture of widely-spaced, rounded ribs; ca. 10 on penultimate whorl, ca. 8 obsolete, "C"-shaped ribs on last whorl, not including varix, with numerous 2° axial threads. Intersections of axial and spiral sculptured with strong, elongated nodules, strongest at periphery. Terminal varix weakly-developed, somewhat constricted, narrow. Aperture oval, outer lip with 4 medial teeth. Anal canal deeply indented between two teeth; columellar tooth bifid. Parietal wall erect with 7 weak lirate teeth. Siphonal canal moderately short, open. Color white with orangish-tan interaxial spaces cut by a white subperipheral narrow band; the spaces form broken flammulations below this band. Aperture white. Operculum, radula, and anatomy unknown.

Figure 264. Distribution of *Cumia clavula* new species (solid), *Cumia sunderlandi* (Petuch, 1995) (bullseye), and *Hesperisternia itzamnai* new species (Z).

Holotype: UF 170226.

Type Locality: 180 m, NE of Contoy Light, Isla Contoy, Quintana Roo State, Mexico.

Paratype: UF 170226.

Distribution: Known only from the type locality.

Habitat: Both shells are worn, collected from 200 m. Substrate unknown.

Etymology: Mayan, *Itzamná*, the creator deity in Mayan mythology. This species is known from off the Yucatan Peninsula, ancestral home of the Mayans. A masculine name.

Discussion: This is apparently a very rare species. Conchologically, it is nearest to *H. jugosa* (Adams, 1852) from the eastern Pacific and *H. janowskyi* (Coltro, 2005) from Brazil, and less so to *H. karinae* (Usticke, 1953) from Brazil. It differs from those species in lacking denticles on the columella, its coloration, and its geographic isolation.

Colubrariidae Dall, 1904

Discussion: The family Colubrariidae has had an uncertain systematic history. Various authors have placed it in the Buccinidae, Ranellidae, or its own family. It is characterized by "a thin, noninvaginable proboscis sac in which the retracted proboscis in convolute, a vestigal radula, a glandular mid-esophagus, and a long a long stomach" (Kay, 1979: 271).

Genus *Cumia* Bivona-Bernardi, 1838

Fusus Helbling, 1779 [rejected name, see Petit and Wilson, 1991, and ICZN, 1994].
Cumia Bivona-Bernardi, 1838: 63, 322.

Type Species: *Cumia decussata* Bivona-Bernardi, 1838, by original designation (= *intertextus* Helbling, 1779).

Description: Small to medium-sized, very elongate. Protoconch minute, sometimes angulate, smooth but grading imperceptibly to teleoconch with addition of C-shaped axial ribs. Spire ≫50% of total length. Sculpture reticulate, obsolete in some species. Varices occur on nearly every whorl, aligned or not. Parietal lip adherent for posterior half of its length. Columella without denticles or lirae, sinuous, only slightly angled. Inner lip with numerous small denticles. See Table 1 for comparison with other genera.

Discussion: Members of *Cumia* are very similar to species of *Colubraria* but differ markedly in protoconch details. In *Cumia* the protoconch appears to arise as a tiny, papillate point from the teleoconch; in *Colubraria* the protoconch is conical and much larger. *Cumia* species occur in the Mediterranean Sea (the type species is *C. intertextus*), Australia, eastern Africa, and the eastern and western Atlantic Ocean.

Cumia clavula new species
(Figures 224–227, 264)

Description: Shell 12.4–18.1 mm in length (holotype 18.1 mm in length). Fusiform, the spire ca. 60% the total length. Protoconch of 1.5 smooth, minute, papillate whorls. Teleoconch of 8 whorls, abruptly arising from the protoconch. Teleoconch sculpture of 24–26 rounded or flattened spiral threads, including siphonal canal, with 1–3 intercalated 2° threads. In some specimens the subsutural spiral cord is larger than the remaining cords. Axial sculpture of numerous, low threads, ca. 50 threads on last whorl, 36–60 threads on penultimate whorl; with very fine 2° threads in between. Intersections of axial and spiral sculpture minutely nodulose. Terminal varix well-developed, set back a short distance from outer lip. Previous varices not aligned, one positioned above the terminal varix, others every ¾ whorl. Aperture elongate-oval, with a weak, columellar plication at the siphonal canal, anal canal delimited by weak denticle on outer lip, none on columella. Outer lip with ca. 17 denticles and no lirations within the mouth. Parietal callus thickened, slightly raised. Siphonal canal short, open. Colored tan with a vague pale band below the periphery and vague spots below the suture. Varices white with tan bands, one at the periphery and two on the siphonal canal. Aperture white. Operculum, radula, and anatomy unknown.

Holotype: UF 341080.

Type Locality: Moín Bay, Limon Province, Costa Rica. No habitat or depth information is available.

Paratype(s): BMSM 17973, 1 shell, 13.6 mm, 1.7 m, under coral rubble, Palenque, Dominican Republic (ex GTW); HGL, 1 shell, 14.5 mm, 5 m, under coral rubble, Isla Beata, Dominican Republic.

Other Material Examined: HGL, 1.7 m, under coral rubble, Palenque, Dominican Republic.

Distribution: Known only from Costa Rica and the Dominican Republic.

Habitat: Shallow water (≪ 5 m). Based on freshly dead shells found among coral rubble.

Etymology: Latin *clavula*, shaped like a small club.

Discussion: This is apparently a very rare species. It differs from the only other western Atlantic species, *Cumia sunderlandi* (Petuch, 1995) from Jamaica, in its smaller size, less polished appearance, fewer axial and spiral threads, and less developed and less reflected terminal varix. See Table 9.

Cumia sunderlandi (Petuch, 1995)
(Figures 228–230, 264)

Colubraria sunderlandi Petuch, 1995: 39–40, figs. 7–9.

Description: Average size 18.3 mm in length (min, 17.3; max, 20.0), the holotype being the largest specimen seen. Fusiform; the spire ca. 66% total length. Protoconch of 1.5 smooth, minute, papillate whorls. Teleoconch of 8.5 whorls, abruptly arising from protoconch. Teleoconch sculpture of ca. 37 rounded or flattened spiral threads, including siphonal canal, but 2° threads are nearly as strong as primaries. Axial sculpture of numerous, sharp threads, 53–88 threads on penultimate whorl, last whorl nearly smooth on last half whorl with numerous fine threads (59–70); very fine 2° threads in between. Intersections of axial and spiral sculpture minutely nodulose. Terminal varix well-developed, set back a short distance from outer lip, with a concave area abaperturally placed, slightly reflected. Previous varices aligned or not above terminal varix, less so on earliest whorls, one per whorl. Aperture elongate-oval, denticles or plications on the columella absent or confined to a few weak plications bounding the canals. Outer lip with very weak denticles (13–17) and none or weak lirations within mouth. Parietal callus thickened, raised. Siphonal canal short, open. Colored tan with narrow, white, sutural band and diffuse, tan flammulations over whorl that may be darkest below suture; a faint subperipheral pale band may also be present. Varices white with 3 tan bands or zones. Aperture white. Operculum, radula, and anatomy unknown.

Table 9. Shell characteristics of *Cumia* species.

	Average length mm	# teleoconeh whorls	# spiral cords on last whorl	# axial ribs on penultimate whorl
clavula	14.7 (18.1)	8	24–26	36–60
sunderlandi	18.3 (20.0)	8.5	37	53–88

Holotype: UF 225165.

Type Locality: Montego Bay, Jamaica, under dead coral slabs in 20 m depth.

Paratypes: Sunderland coll., 2 shells, size not stated, from type locality?

Other Material Examined: Jamaica. HGL, Tyrall, Montego Bay.

Distribution: Known only from Montego Bay, Jamaica.

Habitat: In 20–30 m depth. Apparently known only from freshly dead material. Substrate unknown.

Etymology: Named for Kevan Sunderland, collector of the type material.

Discussion: A very rare species currently only known from Montego Bay. See *Cumia clavula* new species for a comparison with that species. Also see Table 9.

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Empirical estimates of reproductive isolation among the *Physa* species of South Carolina (Gastropoda: Pulmonata: Basommatophora)

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ABSTRACT

Previously published mtDNA sequenee data have suggested that an undeseribed speeies of *Physa* ("Speeies A") may inhabit the swamps and ditches in the southeastern Atlantic eoastal plain. These snails are characterized by slender shells and dark bodies, but are otherwise similar to the more widely distributed *P. pomilia*. Mate ehoice tests revealed signifieant sexual isolation between Speeies A and *P. pomilia*, with homogametie pairings of *P. pomilia* five times more frequent than heterogametie. A set of no-choice outcross experiments yielded only self-fertilized progeny from the Speeies A parent and reproduetive failure from the *pomilia* parent, suggesting complete Species A x *pomilia* hybrid inviability. The third speeies of *Physa* inhabiting South Carolina, *P. acuta*, is more genetieally similar to Speeies A but bears a distinetive penial anatomy. Mate ehoiee tests uneovered no evidence of sexual isolation between Species A and *P. acuta*, and hybridization oecurred readily, with some reduction in parental feeundity but normal F1 viability. Speeies A × *acuta* F1 hybrids appear, however, to be 100% sterile. Thus, the relationship between the degree of reproduetive isolation and genetie divergence seems to be stronger than that between reproductive isolation and penial anatomy in the physid snails of South Carolina. *Physa* Species A warrants formal description.

Additional keywords: Speeiation, *Physella*, *Physa acuta*, *Physa pomilia*, mate ehoiee, sexual isolation, hybridization, allozyme electrophoresis

INTRODUCTION

In recent years, a great deal of interest has foeused on the evolutionary biology of freshwater pulmonate snails in the family Physidae (Tsitronc et al., 2003; Bousset et al., 2004; Henry et al., 2005; 2006; Escobar et al., 2007). Their great reproductive plasticity, which includes selfing, mixed-mating, and outcrossing in either or both sexual roles, together with their ease of culture and the availability of genetic markers, has made physid snails a favorite model for the study of sex allocation generally (Dillon and Wethington, 1992; Wethington and Dillon, 1991; 1993; 1996; 1997). But despite great advanees in our understanding of broad aspects of their reproduetive biology, progress in disentangling the eomplex evolutionary relationships within the family Physidae has been slow.

The elassification system of George Te (1978; 1980) reeognized about 40 speeies and subspecies of physids in North America, arranged into genera and subgenera by penial anatomy. Within the group of nominal species bearing the penial complex Te characterized as "type-b," however, Dillon and Wethington (2006a) reported no reproduetive isolation among *P. gyrina* (Say, 1821), and five other more reeently described speeies: *P. ancillaria* (Say, 1825), *P. aurea* (Lea, 1838), *P. microstriata* (Chamberlain and Berry, 1930), *P. parkeri* (Currier in DeCamp, 1881), and *P. utahensis* (Cleneh, 1925). The addition of *P. sayi* (Tappan, 1838) to the list of type-b synonyms of *P. gyrina* was suggested by the survey of genetie variation at allozyme-encoding loci offered by Dillon and Wethington (2006b).

In the group of physids bearing Te's "penial eomplex type-c," Dillon et al. (2002) could find no reproduetive isolation among *P. integra* (Haldeman 1841) from the American northeast, *P. heterostropha* (Say, 1817) from the American southeast, or the eosmopolitan *P. acuta* (Draparnaud, 1805), described from Europe prior to any Ameriean species of physid. *Physa cubensis* (Pfeiffer, 1839), from the Caribbean, and *P. virgata* (Gould, 1855), from the American West, have also reeently been synonymized under *P. acuta* (Paraense & Pointier, 2003; Dillon et al., 2005). Reproduetive isolation is complete, however, between physids bearing type-b and type-c penial complexes (Dillon et al., 2004).

Te also reeognized a group with penial anatomy intermediate between type-b and type-e. These "type-be" species included *P. hendersoni* (Clench, 1925), originally described as a subspecies of *P. pomilia* (Conrad, 1834). But since Te's observations suggested to him that *P. pomilia* bore type-e penial morphology, he lowered *pomilia* to subspecifie status under *P. heterostropha* and

raised *hendersoni* to the rank of species. More thorough observations and experiments have confirmed, however, that topotypic *P. pomilia* bear penial anatomy type-bc, and that they are not reproductively isolated from *P. hendersoni*, a junior synonym (Dillon et al., 2007).

Recently a new classification has been proposed synthesizing laboratory experiments on reproductive isolation together with mtDNA sequence divergence and morphological observations (Wethington, 2004; Wethington and Lydeard, 2007). This classification recognizes approximately 12 North American species and documents a loose correspondence between mtDNA sequence phylogroups and Te's penial morphologies as outlined above.

In addition, the sequence data of Wethington and Lydeard suggests that a previously unrecognized species of *Physa*, characterized by a dark body and elongated shell, might inhabit the swamps and ditches of the southeastern coastal plain. This species, bearing the type-be penial anatomy of *P. pomilia* but genetically more similar to *P. acuta*, was referred to as "*Physa* Species A". The purpose of the present paper is to report the results of experiments designed to test for reproductive isolation between *Physa* Species A and populations of the two other physids occurring in South Carolina, *P. acuta* (type-e) and *P. pomilia* (type-bc).

The origin and evolution of reproductive isolation has been the subject of intense interest since the early twentieth-century birth of the Modern Synthesis (Mayr, 1942; 1963). The barriers that may evolve between a pair of populations are conventionally divided into prezygotic components (such as sexual isolation) and postzygotic components (such as hybrid inviability or sterility). The former is typically assessed using mate choice tests (Bateson, 1983) and the latter by no-choice breeding experiments (Coyne and Orr, 2004). Here we report the results of both mate choice and no-choice breeding experiments between a reference population of *Physa* Species A from South Carolina and *P. acuta*, then (separately) *Physa* Species A and *P. pomilia*.

MATERIALS AND METHODS

The *Physa acuta* population used to found "line A" for these experiments inhabits the main pond at Charles Towne Landing State Park, west of the Ashley River, within the city limits of Charleston, SC (32.8062° N, 79.9862° W). Snails of this population are not reproductively isolated from *P. acuta* sampled near the type locality for the species in France (Dillon et al., 2002). The *Physa pomilia* population used here to found "line H" was collected from the type locality for *Physa pomilia hendersoni* (Clench, 1925): the Combahee River at the US 21/17A bridge, 1 km E of Yemassee, Hampton County, SC (32.7060° N, 80.8281° W). Dillon et al. (2007) reported no reproductive isolation between this population and snails sampled from Conrad's (1834) type locality for *Physa pomilia* sensu stricto in Alabama. The reference population of *Physa* Species A used to found line "S" was collected from the spring by Huger Creek at Huger Landing, 4 km N of Huger, Berkeley County, South Carolina (33.1305°N; 79.8111°W).

All snails were cultured in transparent polyethylene 10 ounce drinking cups filled with approximately 210 ml of aerated, filtered pond water and covered with a 95 x 15 mm polystyrene Petri dish lid. They were fed O.S.I. Spirulina Aquarium Flake Food, sold in pet stores primarily as a diet for herbivorous aquarium fishes. All experiments took place at room temperature, approximately 23°C. I initially isolated ten wild-collected snails from each study population in separate cups, collected egg masses with weekly water change, and reared the offspring to 2 mm shell length, approximately 3 weeks post-hatching (well in advance of maturity). These three sets of wild-collected but laboratory born sibships were designated A1 through A10, S1 through S10, and H1 through H10. From these sibships were drawn isolates for the mate choice tests and pairs of parents for the study of postzygotic reproductive isolation.

For mate choice tests, large samples of juvenile snails from all three populations were reared to maturity over the course of 8–10 weeks isolated in individual cups, with weekly feeding and water change. Two experiments were performed: one comparing Species A to *P. acuta* and the other comparing Species A to *P. pomilia*. Each experiment was composed of three trials, each trial involving 10 adult snails from one population and ten adult snails from a second, all approximately matching in their shell sizes. Snails were blotted dry and marked with a small dab of fingernail polish according to their population of origin. Then the 20 individuals were simultaneously introduced into a 2 liter glass beaker (filled with 1,400 ml of filtered, aerated pond water) and placed on a glass table to facilitate observation.

Mating activity was monitored for 6 hours. When a snail first successfully copulated as male (defined as the complete insertion of its penis into the gonopore of a partner) it was removed from the beaker, its shell marked with a dot of white correction fluid, and returned. Each individual was often involved in many matings over the 6 hours of observation, both in the male and in the female role, but only its first successful copulation in the male role was recorded. This was an arbitrary decision on my part (since both copulants in a pair might mate in either role, and the result is not a "choice" but rather the outcome of a contest), but necessary nevertheless to prevent double-counting. Note that this design yields a slight bias toward heterogametic pairings, not 1:1 but rather 9:10.

Each trial involved 20 fresh snails, entirely unmated. Three such trials were performed testing for sexual isolation between Species A and *acuta* (the SA experiment) and three additional trials performed testing for sexual isolation between Species A and *pomilia* (the SH experiment), pooling results within experiment to yield a maximum of 60 observations in each case. Chi-square statistics were calculated from the pair of 2 x 2 contingency tables that resulted, normalized by 4/N, as a measure of sexual isolation (Gilbert and Starmer, 1985).

For no-choice tests of postzygotic reproductive isolation, three sets of incross control cups were established using pairs of unrelated parents drawn from the ten sibships within each of the populations (S, H, and A), as for example S1×S2, S2×S3, . . . , S10×S1. Two sets of outcross experimental cups were also established with 10 pairs of snails across populations, the SA cross (S×A1, S×A2, . . . , S×A10) and the SH cross (S×H1, S×H2, . . . S×H10). Each pair of parents received a water change and fresh food every 7 days, at which time the sides of the cup were inspected for egg masses. (Note that any egg mass might result from outcrossing, or be the product of self-fertilization by either parent.) If egg masses were present, all embryos were counted and adults transferred to a fresh cup. Eggs were monitored until hatching (generally about 2 weeks) and all viable, crawling F1 juveniles counted. Observation was terminated upon the death of either parent in a pair.

Crosses were initiated with pairs of snails aged one week post hatch. Then any difference in the central tendency of age at first reproduction (in weeks post hatch) between the 10 outcross pairs and the combination of both sets of 10 corresponding control pairs was tested by calculating a combined (30 pair) median and comparing counts above and below that median using Fisher's exact tests.

For statistical analysis of fecundity and F1 viability, week 1 was established separately for each set of 10 pairs as the first week in which eggs were laid by 3 or more pairs of parents. Embryos and viable hatchlings were subsequently counted for 10 weeks. I then averaged the embryo production of each pair of parents across its lifetime, ignoring any leading (pre-maturity) zeros and any postmortem zeros, while including as 0 any failure to reproduce by viable, mature pairs. So, for example, if one parent in a pair of snails died at week 6, leaving a record of 0, 0, 40, 0, 50 embryos for the pair, their mean fecundity would be 90/3 = 30 embryos per week. A Kruskal-Wallis nonparametric ANOVA was used to test whether any significant difference existed in the central tendency of weekly mean fecundity of either set of 10 outcross pairs (SH or SA) and the 2 corresponding sets of 10 control pairs.

Similarly, I averaged the counts of F1 hatchlings within pairs across weeks, ignoring zeros not corresponding to embryo production, and divided each pair mean by its mean embryo production to obtain pair mean F1 viability. If 35 + 45 hatchlings were recovered from the example pair of snails above, their mean F1 viability would be (35/40 + 45/50)/2 = 88.9%. A second Kruskal-Wallis nonparametric ANOVA was used to test whether any significant difference existed in the central tendency of weekly mean F1 viability posted by either set of 10 outcross pairs and its 2 corresponding sets of 10 control pairs.

To assess the fertility of putative hybrid offspring, F1 hatchlings (from both experimental sets and all three control sets) were reared from each of 3 separate unrelated pairs to size 2 mm. These were crossed in time series: 1 early pair from eggs laid around week 1, 1 middle pair produced around week 5, and 1 late pair produced around week 10, to yield 9 F1 pairs. So if the putative hybrid progeny were reared from pairs S×A1, S×A2, and S×A3, for example, they were crossed as SA1×SA2 early, SA2×SA3 early, SA3×SA1 early, SA1×SA2 middle, SA2×SA3 middle, . . . , SA3×SA1 late. Nine crosses were likewise constituted for corresponding controls S and A, and the total of 3 × 9 = 27 crosses of F1 snails reared to adulthood for each experiment, with weekly feeding and water change. An identical set of 27 cups was established to evaluate hybrid fertility in the SH experiment. I recorded the dates at which embryos and viable F2 hatchlings were produced by each pair.

A larger sample of F1 progeny from 3 outcross pairs from both the SA and SH experiments were reared to 4–5 mm shell length, at which time they were frozen in 100 µl of tissue buffer for analysis by allozyme electrophoresis. We have identified 12 enzyme-encoding loci at which allozyme variation is interpretable as the product of codominant alleles segregating in Mendelian fashion (Dillon and Wethington, 1994). These are aconitase (Acon), esterases (three loci: Est1, Est3, Est6), glucose phosphate isomerase (Gpi), isocitrate dehydrogenase (two loci: Isdh1 and Isdh2), leucine aminopeptidase (Lap), mannose phosphate isomerase (Mpi), phosphoglucomutase (two loci: Pgm1 and Pgm2), and 6-phosphogluconate dehydrogenase (6pgd). We used horizontal starch gel electrophoresis in an aminopropylmorpholine pH 6 buffer system to resolve allozyme variation at the Gpi, Isdh, and 6pgd loci, a Tris-Citrate pH6 buffer system for Acon, Mpi, and Pgm, and a TEB8 system for 6pgd, Lap, and Est. Details regarding our electrophoretic methods, including a description of our equipment and recipes for stains and buffers, have been previously published (Dillon, 1992; Dillon and Wethington, 1995).

The set of no-choice mating experiments described above were conducted simultaneously with those of Dillon et al. (2007), using identical techniques. The data reported here on the reproductive performance of the A and H incross control lines have been published previously, although the SA and SH experimental results, as well as the S incross control, are original to the present investigation.

RESULTS

The SA mate choice experiments did not reveal any evidence of sexual isolation between Species A and *P. acuta* (Table 1, upper). A total of 49 copulations were observed (of a possible 60 total), apparently without regard to species (normalized $\chi^2 = 0.82$, $p = 0.37$). The SH experiments did, however, suggest prezygotic reproductive isolation between Species A and *P. pomilia* (Table 1, lower). The 38 copulations observed in the SH mate choice tests included only 2 of *pomilia* inseminated by a Species A partner, while 10 *pomilia* were inseminated by *pomilia* partners. There was also a bias toward homozygotic pairings on the Species A side, yielding a

Table 1. Copulations observed in the two mate choice experiments, *Physa* Species A × *P. acuta* (above) and *Physa* Species A × *P. pomilia* (below).

		Males		
		Homogametic	Heterogametic	Totals
Females	Species A (S)	15	15	30
	P. acuta (A)	12	7	19
				49
Females	Species A (S)	16	10	26
	P. pomilia (H)	10	2	12
				38

significant overall deviation from random mating (normalized $\chi^2 = 6.63$, $p = 0.01$).

Reared together in a no-choice design, mixed pairs of Species A and *P. acuta* showed no delay in age at first reproduction, their modal age at maturation (7 wks) indeed slightly less than that observed in either matched Species A or matched *acuta* control pairs (Table 2). A reduction was apparent in parental fecundity, however, the median of 55.2 embryos/wk posted by SA outcross pairs significantly below both controls ($p = 0.027$). The 73.1% median viability of the F1 Species A / *acuta* hybrids was intermediate between the F1 viabilities observed from incross controls.

Electrophoretic analysis of a sample of offspring from three SA outcrosses confirmed the hybridity of all F1 progeny. One pair of parents was fortuitously fixed for alternative alleles at the Isdh locus, yielding a sample of twelve entirely heterozygous progeny. A second pair of SA parents were both heterozygous at the Est3 locus ($Est3^{100}/Est3^{96} \times Est3^{96}/Est3^{92}$), yielding twelve F1 progeny in four classes. The third pair of parents included one heterozygote at the Isdh locus ($Isdh^{100}/Isdh^{100} \times Isdh^{100}/Isdh^{97}$), and one heterozygote at the Est3 locus ($Est3^{100}/Est3^{92} \times Est3^{92}/Est3^{92}$), yielding at both loci twelve F1 progeny representing the heterozygous and one homozygous class, missing the other homozygous class entirely. The likelihood of missing a single homozygous class in twelve selfed progeny from a heterozygous parent would be 0.032.

None of the nine pairs of F1 progeny from the SA outcross produced viable F2 offspring. One SA pair was terminated early by mortality, while the other eight pairs all laid eggs profusely, beginning at week 7 and extending to week 19. All egg masses laid by all eight pairs of SA hybrids over the 12 week period were held for five weeks, with no hatching observed.

Reared together in a no-choice design, pairs of Species A and *P. pomilia* demonstrated significant delays in age at first reproduction behind that posted by their combined controls (Fisher's exact $p = 0.003$). Their modal age of 9 weeks at the onset of egg laying was slightly behind both the Species A control and the *pomilia* control (Table 3). The median parental fecundity of 27.3 embryos/wk posted in the SH outcross experiment was also significantly lower than both incross controls ($p = 0.002$), and the median viability of their progeny (64.8%) lower than the Species A control. Only one of the nine pairs of first generation progeny from the SH experiment yielded viable second generation offspring, at week 20. Most of the remaining first generation pairs laid eggs that failed to hatch, generally over many weeks of observation.

Electrophoretic analysis revealed that two sets of SH parents were fortuitously fixed for alternative alleles at the LAP locus. Samples of ten first generation progeny from both of these crosses yielded only one homozygous class, strongly suggesting self-fertilization by the Species A parent, and no reproduction by the *pomilia* parent. Absence of suitable genetic markers made inference regarding the third set of SH progeny analyzed equivocal.

Table 2. Statistics comparing the fitness of *Physa* Species A × *P. acuta* outcrosses to pure *Physa* Species A and pure *P. acuta* controls.

	Species A	SA outcross	*P. acuta*
First oviposition, P generation			
Mode (weeks post hatch)	8	7	8
Range	7–8	7–10	5–10
Weekly mean parental fecundity			
Median (embryos)	75.4	55.2	66.9
Range	19–105	18–81	17–104
Weekly mean F1 viability			
Median (%)	80.5	73.1	61.7
Range	66–91	49–99	45–86
F1 Fertility	67%	0%	100%
Viable F2 hatch			
Median (week)	12.5	–	9
Range	4–19	–	9–11

Table 3. Statistics comparing the fitness of *Physa* Species A × *P. pomilia* outcrosses to pure *Physa* Species A and pure *P. pomilia* controls.

	Species A	SH outcross	*P. pomilia*
First oviposition, P generation			
Mode (weeks post hatch)	8	9	7
Range	7–8	8–12	7–11
Weekly mean parental fecundity			
Median (embryos)	75.4	27.3	57.4
Range	19–105	7–41	19–68
Weekly mean F1 viability			
Median (%)	80.5	64.8	55.8
Range	66–91	10–86	33–86
F1 Fertility	67%	10%	78%
Viable F2 hatch			
Median (week)	12.5	20	5
Range	4–19	–	3–8

DISCUSSION

The experiments reported here confirm reproductive isolation between the "*Physa* Speeies A" of Wethington (2004) and populations representing both of the other physid species inhabiting South Carolina, *P. acuta* and *P. pomilia*. An initiative to formally describe Speeies A has just been published (Wethington et al., 2009). The reproductive isolation displayed by these three speeies is of different degrees, however, and apparently more elosely related to their genetic divergence than to their reproductive anatomy.

Physa Speeies A and *P. acuta* eluster in the same mtDNA phylogroup (Wethington and Lydeard, 2007) but differ in their penial anatomy. The mate choice tests reported here yielded no evidenee of sexual isolation between them. A significant reduction in the joint feeundity of Speeies A × *P. acuta* outeross pairs was indeed revealed by no-ehoiee breeding experiments, although there was no evidenee of reduced viability in the F1 hybrids sueh crosses produced. Species A × *acuta* hybrids were, however, entirely sterile.

Physa Specics A and *P. pomilia* share identical type-bc penial anatomy, but are more distantly related geneticall-y. The reproductive isolation that Species A and *pomilia* display under eontrolled eonditions is of a greater degree than that observed between Speeies A and *acuta*. Paired in a no-choiee design, Speeies A and *pomilia* parents displayed delayed reproduetion, reduced fertility, and redueed offspring viability. All the viable first-generation progeny recovered from SH outerosses were attributable to self-fertilization by the Speeies A parent, reproduction by the *pomilia* parent apparently foreclosed. It seems likely that the substantial reduetion in reproductive sueeess demonstrated in the second generation by SH offspring may be attributable to Species A inbreeding depression. Sueh results are quite simiar to those we obtained from crosses between the type-e *Physa acuta* and the type-b *Physa gyrina* (Dillon et al., 2004).

In addition, mate choice tests returned evidence of prezygotie reproduetive isolation between Species A and *P. pomilia*. Physids seem to mate aecording to a modified "Bateman's Principle" (Bateman, 1949). They are generally quick to eopulate as males, and display little discrimination, but when mounted in the female role they can be choosy, often displaying rejective behaviors like evasion and shell-shaking (DeWitt, 1991; 1996; Wethington & Dillon, 1996; McCarthy and Sih, 2008). Only 38 of the 60 snails tested in the SH mate choice experiments were ultimately able to mate as males, at least partly because of the high frequency of rejectivc bchaviors they eneountered in heterogametic couplings. Our observation that *pomilia* eopulants were more rejective of heterogametic insemination than Species A copulants may be related to our separate observation that the Species A partner in our SH no-choice experiments retained the ability to reproduce by self-fertilization, while the *pomilia* partner apparently did not. This situation is similar to that we have previously deseribed for the interspecifie pairing of *Physa acuta* and *P. pomilia* (Dillon et al., 2007).

Although nothing is known about the geneties of reproductive isolation in pulmonate snails, a large body of research has eonfirmed that both prezygotie and postzygotic barriers are inherited in a eomplex and polygenic fashion in *Drosophila* (Wu and Palopoli, 1993; Ritehie and Phillips, 1998). In general it has been found that postzygotie isolating mechanisms evolve independently of, and tend to lag behind, prezygotie meehanisms (Coyne and Orr, 2004). Whether the latter ean be reinforeed by natural seleetion on the former is controversial. Experiments to traee the evolution of both sets of eharacters through the larger phylogeny of the Physidae are eurrently ongoing.

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Genetic and morphological characterization of the Physidae of South Carolina (Gastropoda: Pulmonata: Basommatophora), with description of a new species

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ABSTRACT

Recent experimental studies of reproductive isolation have distinguished three physid species in South Carolina: the cosmopolitan *Physa acuta*, bearing a one-part penial sheath, and two more restricted species bearing subdivided penial sheaths: *Physa pomilia* and *Physa* "Species A." Here we describe "Species A" as *Physa carolinae*, an inhabitant of floodplain swamps and ditches of a vernal or intermittent character, ranging through coastal plain and lower piedmont regions from Virginia to Florida. *Physa carolinae* may be distinguished from *P. pomilia* by its larger adult size, more slender and elongate shell, and uniformly dark pigmentation. A sample of 11 *P. carolinae* from five South Carolina populations averaged greater than 10% sequence divergence from standard populations of *P. acuta* and *P. pomilia* for both CO1 and 16S mitochondrial genes. The circumstances under which a widespread and seasonally abundant freshwater gastropod such as *P. carolinae* might escape scientific notice for almost 200 years are reviewed.

Additional keywords: Taxonomy, Phylogeny, Gastropoda, *Physella*, *Physa acuta*, *Physa pomilia*, mtDNA sequence, CO1, 16S.

INTRODUCTION

Pulmonate gastropods of the family Physidae are a common element of the freshwater benthos in South Carolina and throughout North America. Longstanding taxonomic confusion has, however, impeded any real advance in our understanding of their ecology and distribution. The initial monographic review of the family was that of Haldeman (1842), who recognized 12 species in the United States, only one of which ranged into South Carolina, *Physa heterostropha* (Say, 1817). Binny's (1865) monograph included 30 specific physid nomina in two genera (*Physa* and *Bulinus*), three of which might potentially inhabit South Carolina: *Physa gyrina* (Say, 1821) and *Physa ancillaria* (Say, 1825) in addition to *P. heterostropha*. Crandall (1901) recognized as valid only 17 physid species in eastern North America, two of which he admitted to South Carolina: *Physa gyrina* and *P. pomilia* (Conrad, 1834). Only four species were listed as confirmed for the state by Mazyck (1913): *P. gyrina*, *P. pomilia*, *P. heterostropha* and *P. cubensis* (Pfeiffer, 1839). Walker (1918) catalogued 77 specific nomina in the family Physidae of North America, approximately half of which were in synonymy at the time, but did not provide ranges.

The most influential twentieth century monograph of the American Physidae was that of Te (1978; 1980). He recognized approximately 40 species and subspecies, classified by penial morphology into four genera: *Physa* (sensu stricto), *Physella*, *Aplexa*, and *Stenophysa*. Te (in Burch, 1989) listed three species whose range might include South Carolina: *Physella gyrina* (with several subspecies), *Physella hendersoni* (Clench, 1925), and *Physella heterostropha pomilia*.

Recent studies of genetics, morphology, and reproductive biology have shown, however, that the number of valid North American species in the family Physidae has been overestimated. Wethington and Lydeard (2007) have proposed a return to the two-genus classification of the Physidae, *Aplexa*, and *Physa*, the former with one North American species and the latter with approximately ten. *Physa heterostropha* and *P. cubensis* have been shown to be junior synonyms of the cosmopolitan *P. acuta* (Draparnaud, 1805), and *P. hendersoni* a junior synonym of *P. pomilia* (Dillon et al., 2002; Paraense and Pointier, 2003; Wethington, 2004; Dillon et al., 2007; Wethington and Lydeard, 2007). No populations of bona fide *P. gyrina* have been confirmed from South Carolina (unpublished observations).

During preliminary surveys of mtDNA sequence divergence among South Carolina populations of *Physa acuta*, Wethington (2004) distinguished a population of *Physa* from Johns Island (Charleston County) bearing elongate shells and dark bodies. This population, previously referred to *Physa heterostropha pomilia* ("JNI") by Dillon and Wethington (1995), was phylogenetically distinct from known *P. acuta* controls, with a genetic

distance between 14.5–18.9% (combined mtDNA 16S +CO1, without loops and truncated). Additional populations bearing similar morphology and mtDNA haplotypes were identified and referred to "*Physa* species A" by Wethington and Lydeard (2007).

Controlled breeding experiments have recently confirmed reproductive isolation between "*Physa* Species A" and both *P. acuta* and *P. pomilia* (Dillon, in review). In this paper we describe "Species A" as *Physa carolinae* and distinguish it both morphologically and genetically from *P. acuta* and *P. pomilia*, which themselves have been confused and poorly characterized in some respects.

MATERIALS AND METHODS

Study Populations: Our reference population of *Physa acuta* was sampled from the main pond at Charles Towne Landing State Park, within the city limits of Charleston, South Carolina (32.8062°N, 79.9862°W). Breeding experiments have shown this population to be conspecific with near-topotypic *P. acuta* from France (Dillon et al., 2002). This population has previously been designated "Ctl" by Dillon and Wethington (1995), population "C" by Dillon et al. (2005) and Wethington (2004), and population A by Dillon et al. (2004, 2007) and Dillon (in review). The habitat has been described by Dillon and Dutra-Clark (1992).

Our reference population of *Physa pomilia* was collected from the Combahee River at the US 21/17A bridge in Yemassee, South Carolina (32.7060°N; 80.8281°W). This site was given as the type locality for *Physa pomilia hendersoni* by Clench (1925). Dillon et al. (2007) reported no reproductive isolation between the Yemassee population (population H) and snails sampled from Conrad's (1834) type locality for *Physa pomilia* in Alabama. Reproductive isolation is complete, however, between population H and both *P. acuta* and Species A (*P. carolinae* new species) (Dillon, in review). This population was designated "*ysr*" by Wethington (2004) and "*scysr*" by Wethington and Lydeard (2007).

Our reference population of Species A (*P. carolinae* new species) was sampled from a spring by Huger Creek at Huger Landing, 4 km N of Huger, Berkeley County, South Carolina (33.1305°N, 79.8111°W). This is the same population from which Dillon (in review) founded the line "S" for studies of reproductive isolation. For mtDNA sequence analysis we sampled five additional populations from South Carolina, as follows. Population jni was collected from agricultural ditches 3.5 km NE of Legareville, Johns Island, Charleston County (33.1305°N, 79.8111°W). This is the original "JN1" of Dillon and Wethington (1995), also analyzed as "scjni" by Wethington (2004) and Wethington and Lydeard (2007). Population blac was collected from the Black River at the boat ramp near the SC 41 bridge, Williamsburg County (33.4905°N, 79.5459°W). Population bull was sampled from Bull Bridge Creek at SSR 38 bridge, Charleston County (32.8182°N, 80.3994°W). Population hell was sampled from Hellhole Bay Swamp by SC 41, 1.5 km SW of Jamestown, Berkeley County (33.2749°N, 79.7041°W). Population mac was collected from Melichamp Creek near the SC 165 bridge, Charleston County (32.7620°N, 80.2416°W).

Sequencing: DNA was extracted from 36 individual snails: 20 *Physa acuta*, 5 *Physa pomilia*, and 11 *Physa* Species A (*P. carolinae* new species) from five populations (jni = 4, blac = 2, bull = 2, mac = 2, hell = 1). Although all 36 were successfully amplified and sequenced for 16S mtDNA, only a subset of 23 were sequenced for CO1 (14 *P. acuta*, three *P. pomilia*, and six *Physa* Species A (*P. carolinae* new species): 2 jni, 2 bull, 1 blac, 1 hell).

DNA was extracted from whole tissue using standard phenol chloroform procedures (Sambrook et al., 1989). Pieces of mtDNA from genomic DNA were copied and augmented via the Polymerase Chain Reaction using 16S primers (L2510 and H3080=16Sar-L and 16Sbr-H; Palumbi et al., 1991) for a 550 base pair segment and CO1 primers (LCO1490 and HCO2198; Folmer et al., 1994) for a 650 base pair segment, cleaned using standard procedures and then cycle-sequenced. The double-stranded PCR products were generated using 50–500 ng of template genomic DNA in 25 µl volumes (10 mM Tris, 50 mM KCL, 2.5 mM $MgCl_2$, 1 µM of each primer, 0.1 mM of each dNTP, 1.5 units Taq DNA polymerase; Fisher Scientific). The amplification regime began with a denaturation at 92°C for two minutes followed by 35 cycles of the following: denaturation at 92°C for 40 seconds, annealing at 52°C for 60 seconds (16S) or 50°C for 60 seconds (CO1), and extension at 68°C for 90 seconds. The amplified DNA was then concentrated using Millipore Ultrafree MC filters and provided the template for cycle sequencing using the ABI BigDye kit following manufacturer's instructions. The reactions were purified using Quiagen DyeEx spin columns and sequenced on an ABI3100 genetic analyzer.

Sequences were aligned by eye directly for CO1 and by using the LSU rDNA secondary structure for 16S (Lydeard et al., 2000) using BioEdit (Hall, 1999). Loops and indels were excluded from analysis of the 16S data set, lowering the effective sequence length from 533 to 446 base pairs. Two separate phylogenetic analyses were employed: a Bayesian analysis and a Maximum Likelihood analysis.

MrBayes v3.0B4 (Ronquist and Huelsenbeck, 2003) was used for the Bayesian analysis, with posterior probabilities guided by the General Times Reversible model. The CO1 and 16S gene portions were analyzed separately due to limitations in computer memory. There were four separate Monte Carlo Markov chains and the number of generations was preset to 10,000,000 with the first 10,000 generations excluded from the analysis for both runs. The burn in value was sufficient for stable likelihood tree values for each analysis. Probabilities were calculated for each node. Since CO1 is a coding region of the mtDNA genome, a coding block was used. The data were partitioned by codon and the GTR model

applied for each defined partition within the 600 base pair segment used. A non-coding block was used to analyze the 446 bp of the 16S gene, again under the GTR model, to infer the Bayesian phylogeny.

Modeltest (Prosada and Crandall, 1998) was employed to pick the best fitting model for the evolution of base pair substitution for a maximum likelihood analysis. The JC model, equal base frequencies, and all rates equal appeared to be the best model to use for the CO1 gene portion while the HKY + G, K = 5, different base frequencies (A = 0.3678, C = 0.1324, G = 0.1840, and T = 0.3158) with a ti/tv substitution ratio of 0.9928 appeared to be the best model for the 16S gene portion. Since different models were picked, the CO1 and 16S gene portions were analyzed separately.

Morphology: Standard length was measured as the maximum shell dimension on samples of 30 adults from each of the three reference populations. Shell width was measured as the maximum dimension perpendicular to shell length. The significance of the difference in shell width between *Physa* Species A (*P. carolinae* new species) and *P. pomilia* (holding length constant) was tested with analysis of covariance using the separate slopes model (JMP version 7). All 90 of these specimens have been deposited as vouchers in the Academy of Natural Sciences of Philadelphia, 20 as dry shells and 10 in absolute ethanol for each species. The 30 individuals of *Physa* species A constitute the holotype and paratypes of *Physa carolinae* new species.

Initial anatomical observations were made on living snails with a Zeiss dissecting microscope. Shells were then cracked and whole animals dissected and stained with toluidine blue. Line drawings were composed both freehand and with the aid of a camera lucida. Radula were extracted from the buccal mass with a dilute solution of commercial bleach, air-dried, coated with gold-palladium and examined with a JEOL JSL 6000 scanning electron microscope set from 5–10 KV.

RESULTS

Sequence Divergence: A total of 28 unique mtDNA sequences were obtained from the 36 snails we amplified for the 16S gene, and 15 unique mtDNA sequences were obtained from the subset of 23 snails successfully amplified for CO1. Genbank accession numbers are given in Table 1. Bayesian analysis of both data sets confirmed that *Physa* Species A (*P. carolinae* new species), *P. acuta*, and *P. pomilia* were all monophyletic approaching 1.0 probability, all five populations of Species A (*P. carolinae* new species) clustered together quite distinctly from *P. acuta* and *P. pomilia* (Figures 1 and 2). The maximum likelihood analysis of both data sets confirmed the Bayesian analyses (Figures 3 and 4). There appear to be three separate phylogenetic species uncovered in our sampling of South Carolina snails. Both 16S analyses reveal a basal and distinct population ("mac") within the Species A (*P. carolinae* new species) clade.

Table 1. Genebank accession numbers for all individual *Physa* sequenced.

Species	Individual	Genbank 16S	Genbank CO1
Physa acuta	Ctl1	GQ415009	See C14
	Ctl3	GQ415010	See C12
	C1	GQ415011	–
	C2	GQ415012	GQ415033
	C3	GQ415013	GQ415034
	C4	See C11	See C12
	C5	GQ415014	GQ415035
	C6	See C11	See C12
	C9	See C11	GQ415036
	C11	GQ415015	GQ415037
	C12	See C11	GQ415038
	C14	GQ415016	GQ415039
	C15	GQ415017	–
	C16	GQ415018	See C12
	C18	GQ415019	–
	C19	See C11	–
	C21	See C11	See C14
	C23	GQ415020	–
	C24	GQ415021	–
	C27	See C11	See C14
Physa carolinae	blac1	GQ415022	GQ415040
	blac2	GQ415023	–
	bull1	GQ415024	GQ415041
	bull2	GQ415025	GQ415042
	hell1	GQ415026	GQ415043
	jni1	EU038348	EU038395
	jni2	EU038349	EU038396
	jni7	GQ415027	–
	jni11	GQ415028	–
	mac1	GQ415029	–
	mac2	GQ415030	–
Physa pomilia	ysr1	AY651232	AY651194
	ysr2	AY651233	AY651195
	ysr3	AY651234	AY651196
	ysr4	GQ415031	–
	ysr5	GQ415032	–

However, the bootstrap support for this group is weak in the maximum likelihood analysis.

Physa acuta and *Physa* Species A (*P. carolinae* new species) appear to be the most genetically similar species pair by a slight margin. Their 16S sequence divergence ranged from 8.5%–12.6% (uncorrected p-values), with 446 nucleotides in the denominator, and their CO1 divergence ranged from 14.7%–17.1%, with 600 nucleotides in the denominator. Both of these ranges were slightly below those recorded for *P. pomilia* and Species A (*P. carolinae* new species) (16.1–17.7% 16S, 17.5–18.8% CO1), and *P. pomilia* and *P. acuta* (15.5–16.6% 16S, 18.5–20.5% CO1). Within-species percent base pair divergence ranged up to 7.4% for 16S and 13.0% for CO1, both values recorded between individuals sampled from rather distant populations of Species A (*P. carolinae* new species).

Morphometrics: Regressions of shell width on shell length for 30 individuals sampled from each of the three reference populations are shown in Figure 5. The regression equations of Y = 0.42x + 1.1 (r = 0.68) for *P. pomilia*

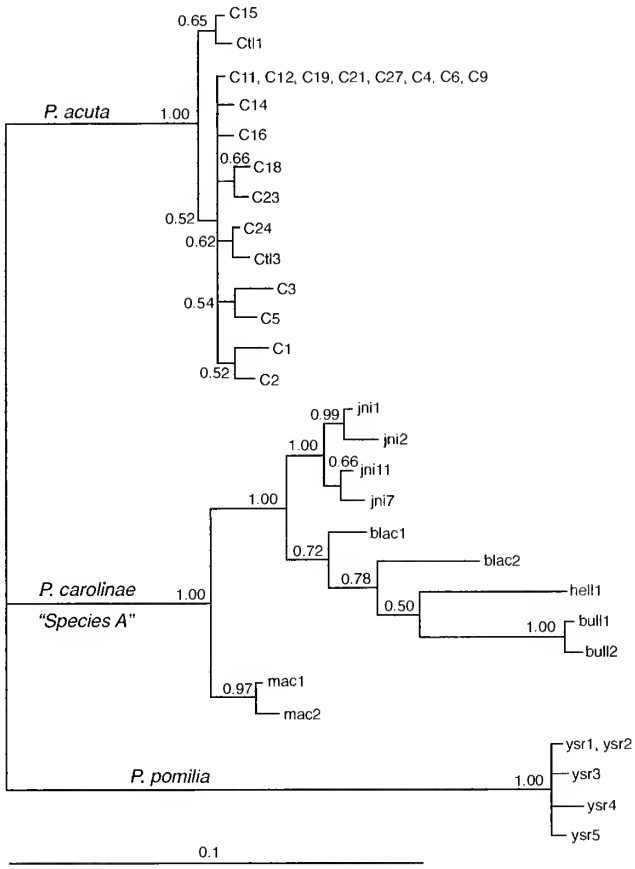


Figure 1. 16S Bayesian analysis showing the three genetically distinct South Carolina species: *Physa acuta*, *P. pomilia*, and *Physa* Species A (*P. carolinae* new species).

and Y = 0.40x + 0.95 (r = 0.69) for Species A (*P. carolinae* new species) demonstrated no significant difference in slope (0.42 ± 0.09 and 0.40 ± 0.08, respectively). Their Y-intercepts were significantly different, however, separate-slopes analysis of covariance returning a value of t = –2.82 (p = 0.007). Thus, while *P. carolinae* bears a more significantly slender shell, the rate at which its whorls expand is similar to that of the anatomically similar *P. pomilia*.

The regression of shell width on shell length for *P. acuta* was Y = 0.71x – 0.48 (r = 0.92). With a slope significantly greater than 0.5 (0.71 ± 0.08), shells of individual *P. acuta* tend to grow wider as they mature, while those of *Physa* Species A (*P. carolinae* new species) and *P. pomilia* tend to grow narrower.

SYSTEMATICS

Family Physidae Fitzinger, 1833
Genus *Physa* Draparnaud, 1801

Physa acuta Draparnaud, 1805
(Figures 1–14)

Physa acuta Draparnaud, 1805: 55, pl. 3, figs. 10–11.
Lymnaea heterostropha Say, 1817: no pagination, pl. 1, fig. 6.
Physa cubensis Pfeiffer, 1839: 354.
Physa integra Haldeman, 1841a: cover, 3. 1842–43: 33, pl. 4, figs. 7–8.
Physa mexicana Philippi, 1841: 5, pl. 1, figs. 3–4.
Physa osculans Haldeman, 1841b: 78, pl. 4, fig 6.
Physa venustula Gould, 1847: 215; 1852: 115, pl. 8, figs. 134–134b
Physa jamaicensis C. B. Adams, 1851:174.
Physa virgata Gould, 1855: 128.
Physa niagarensis Lea, 1864: 114; 1866: 168, pl 24, fig 97.
Physa billingsi Heron, 1880: 62, fig. 5.
Physa conoidea Fischer and Crosse, 1886: 101, pl. 39, figs 8–8a.
Physa lacustris Clessin, 1886: 344, pl. 48, fig. 9.
Physa cupreonitens Coekerell, 1889a: 63; 1889b: 1, fig. 1.
Physa osculans patzcuarensis Pilsbry, 1891a: 9; 1891b: 323, pl. 15, fig. 5.
Physa porteri Germain, 1913: 161, fig. 20.
Physa bottimeri Clench, 1924: 12.
Physa elegans Clench and Aguayo, 1932: 37, Clench 1936: 342, pl. 25, fig. 1.
Physa natricina Taylor, 1988: 67, fig. 6a–n.
Physella winnipegensis Pip, 2004: 42–48; Pip and Frank, 2008: 10–16.

Description: The shell and anatomical morphology have been well-characterized by Paraense and Pointier (2003). Our observations on individuals sampled from

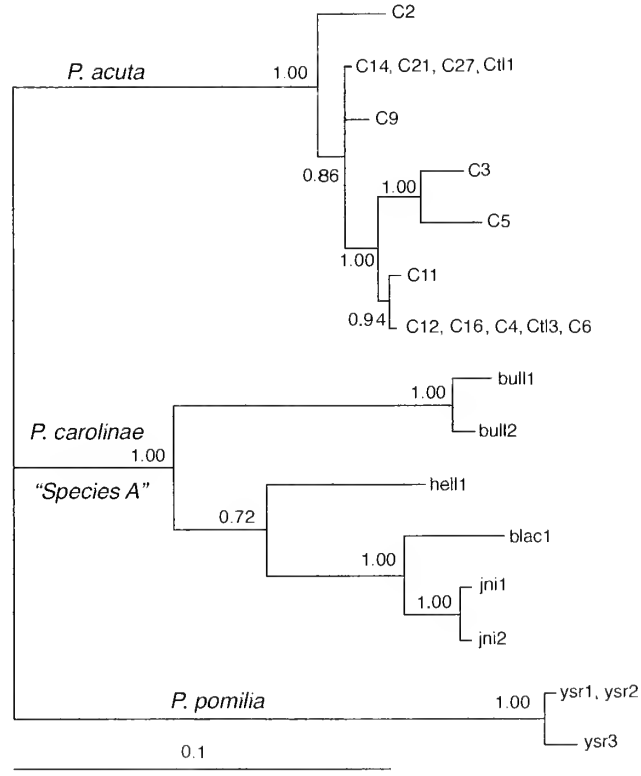


Figure 2. CO1 Bayesian analysis showing the three genetically distinct South Carolina species: *Physa acuta*, *P. pomilia*, and *Physa* Species A (*P. carolinae* new species).

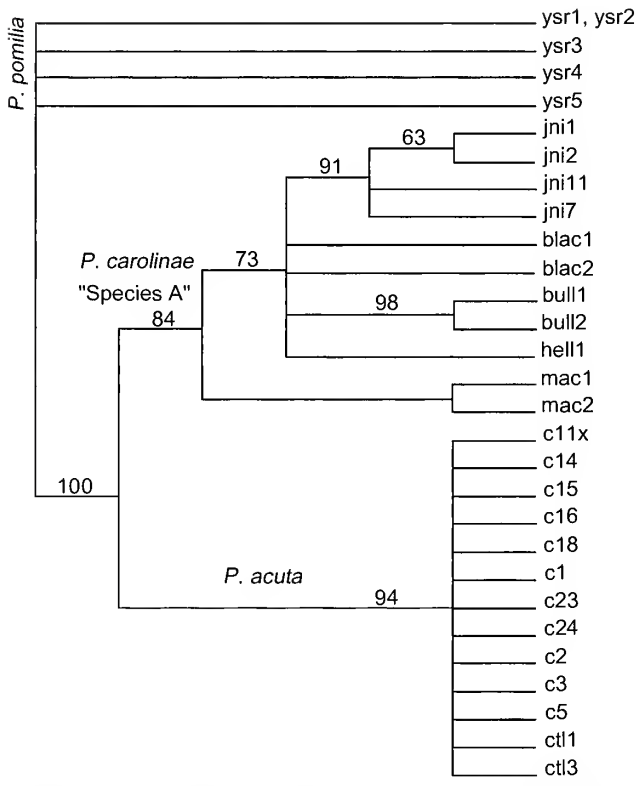


Figure 3. 16S Maximum likelihood analysis using HKY+G model with the following base frequencies: A = 0.3678, C = 0.1324, G = 0.1840, and T = 0.3158 showing three genetically distinct South Carolina species: *Physa acuta*, *P. pomilia*, and *Physa* Species A (*P. carolinae* new species). C11x represents the following identical haplotypes: c11, c12, c19, c21, c27, c4, c6, and c9.

the reference population at Charles Town Landing do not differ in any material respect. Shell (Figure 6) sinistral, elongate-ovate, high spired, thin, translucent, lustrous, with faint spiral growth lines. Body whorl approximately 85% of shell length, with four to five adult whorls, with rounded shoulders and impressed sutures. Spire profile flat to slightly concave, apex sharply pointed ("acute"). Large auricular aperture, approximately 75% of shell length, with thin outer lip. Mature size is reached about 6–8 weeks post-hatch in our standard culture conditions, at mean shell lengths ranging from 5.3–7.4 mm (Wethington and Dillon, 1993; 1997). From the regression shown in Figure 3, the predicted ratio of length to width for a 6 mm individual would be 1.59, and that of an 8 mm individual would be 1.54. Cephalopedal mass (Figure 9) light gray to tan, with long, slender tentacles and rounded or fan-like labial palps. Jaw simple, lacking lateral processes. Mantle typically bearing a reticulate pigmentation pattern, sometimes demonstrating digitations. Foot extending approximately the length of the shell, pointed posteriorly. Penial complex (Figure 11) includes a preputium (with preputial gland) and a muscular (non-glandular) penial sheath. This general penial morphology has been characterized as "type-c" (Te, 1978; Wethington and Lydeard, 2007). When everted, the penis slides through the preputium to form a long, simple, fingerlike projection, with a lateral lobe corresponding to the preputial gland. Radula (Figure 14) comprising approximately 30–40 V-shaped rows of approximately 120–160 comb-like teeth. Each row has a tricuspid median flanked by 60–80 teeth bearing approximately 8–12 cusps.

Synonymy: An extensive synonymy has been published by Taylor (2003). In addition, breeding studies have uncovered no evidence of reproductive isolation between *P. acuta*, *P. heterostropha*, *P. integra*, or *P. virgata* (Dillon et al., 2002; 2005). *Physa cubensis* Pfeiffer was synonymized under *P. acuta* by Paraense and Pointier (2003), and *Physa natricina* Taylor by Rogers and Wethington (2007). The weight of these studies, together with the DNA sequence results of Wethington and Guralnick (2004) and Wethington and Lydeard (2007), combine to suggest the additions to the synonymy of Taylor (2003) listed above.

Vouchers: Academy of Natural Sciences of Philadelphia, 20 dry shells (ANSP 422686) and 10 in 100% ethanol (ANSP A21949).

Type Locality: River Garonne, France.

Distribution and Habitat: Dillon et al. (2002) nominated *P. acuta* as "the world's most cosmopolitan freshwater gastropod," with a modern range extending across six continents. Populations are common throughout South Carolina in ponds, reservoirs, and the margins of

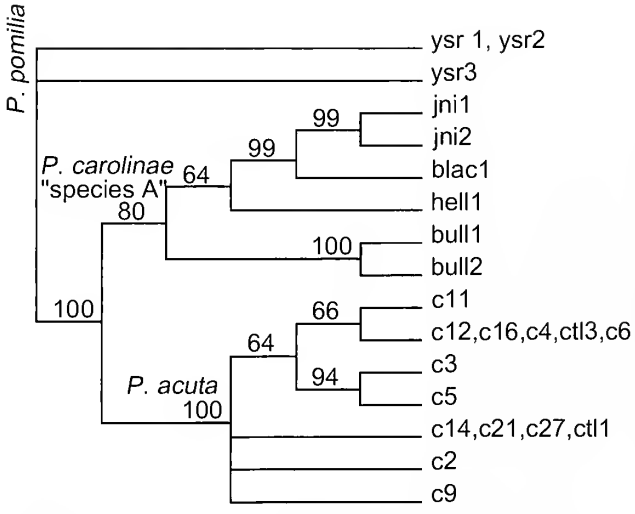


Figure 4. CO1 Maximum likelihood analysis using JC model with equal base frequencies and an equal rate substitution showing the three genetically distinct South Carolina species: *Physa acuta*, *P. pomilia*, and *Physa* Species A (*P. carolinae* new species).

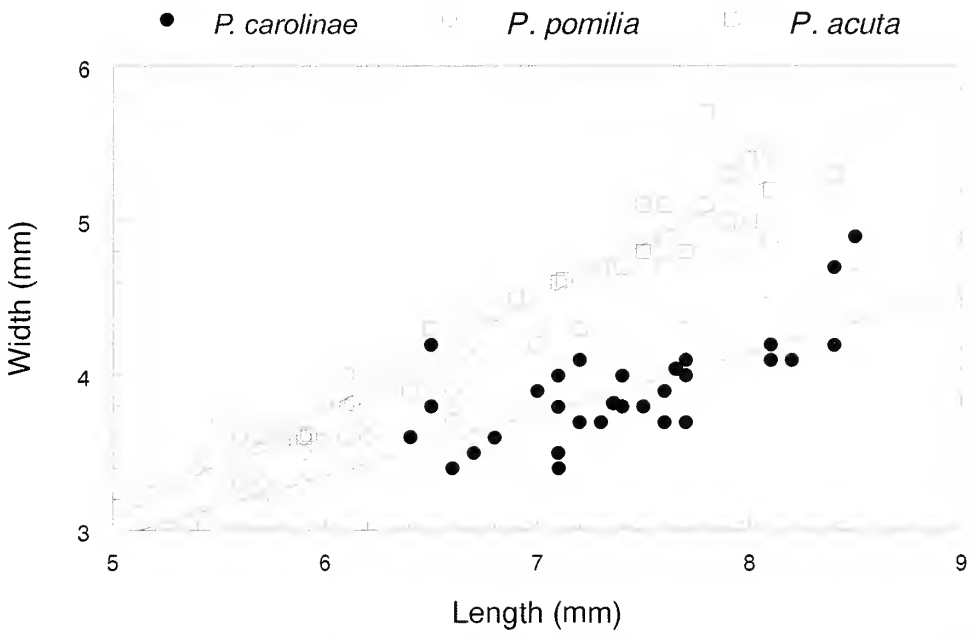
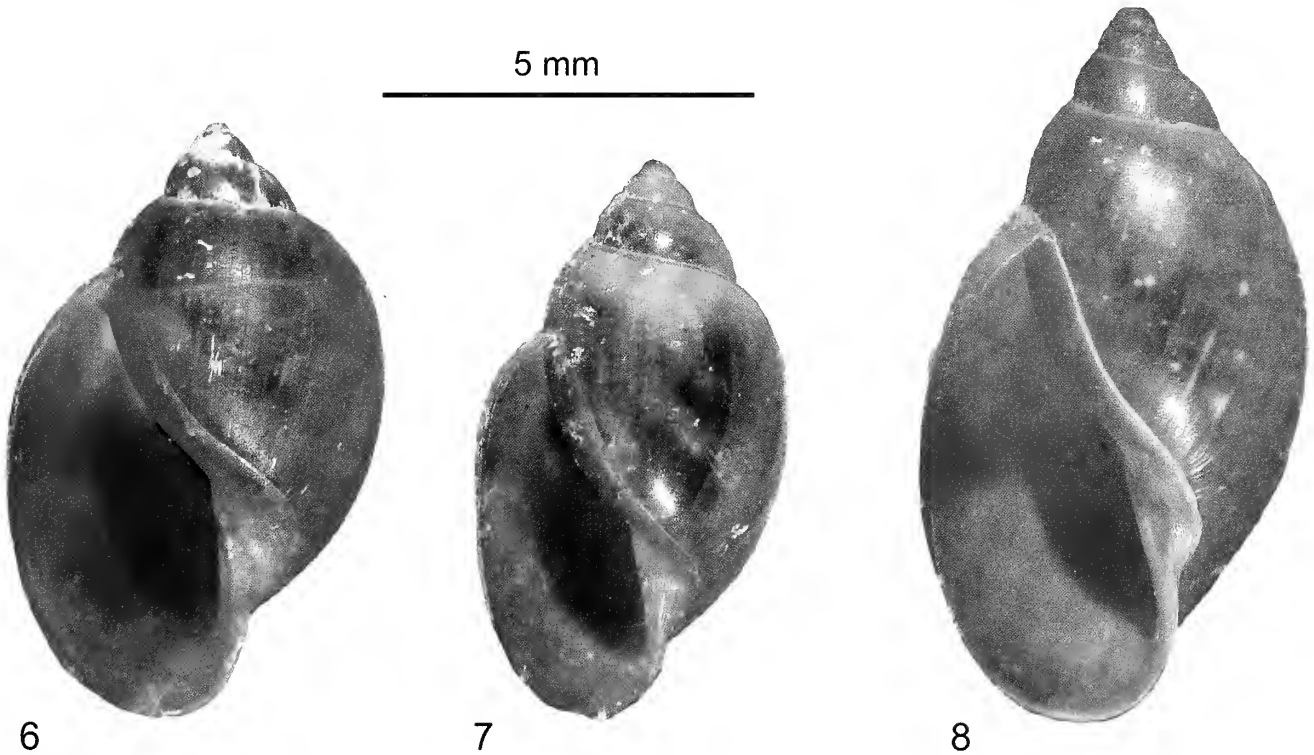


Figure 5. Shell width as a function of shell length in three samples of *Physa* from South Carolina: *Physa carolinae* new species (Species A) (dark circles, lower solid line), *P. pomilia* (open circles, dashed line) and *P. acuta* (squares, upper solid line).

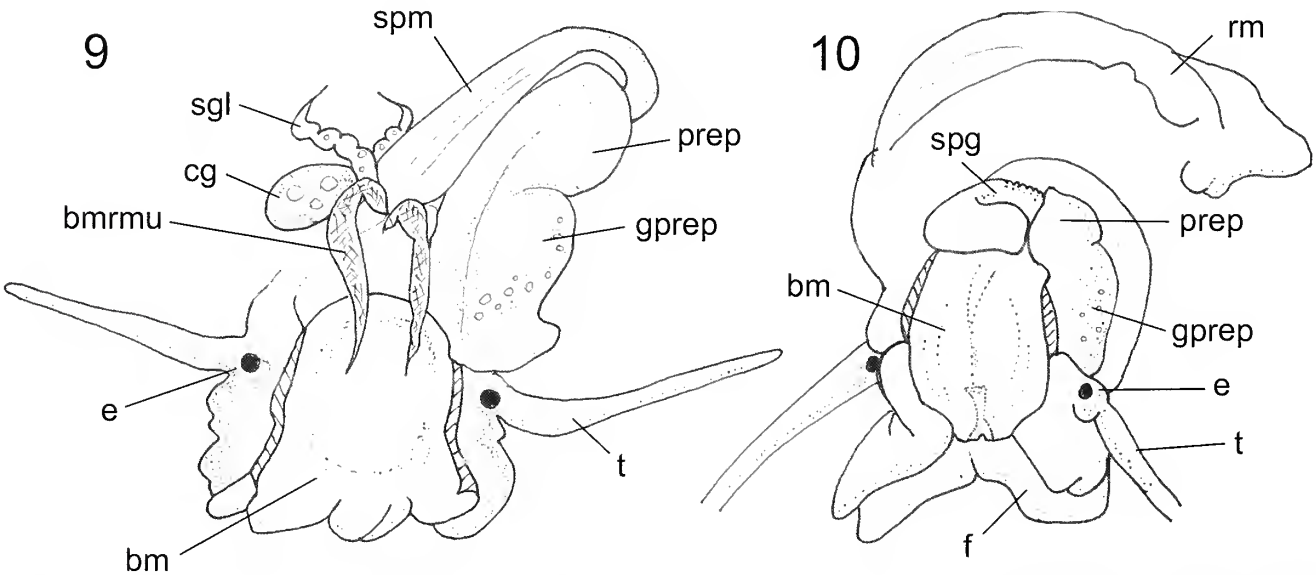
rivers and streams with low current, especially in rich or disturbed environments.

Physa pomilia Conrad 1834
(Figures 1–13)

Physa pomilia Conrad, 1834: 343; 1866: 278, pl. 15, figs. 1–3.
Bulinus pumilus Beck, 1837–38: 117.
Physa showalteri Lea, 1864: 115; 1866: 170, pl. 24, fig. 92.
Physa pomilia arionus Clench, 1925a: 2, pl. 1, fig. 2.



Figures 6–8. Example shells from the three reference populations. **6.** *Physa acuta* (ANSP 422686) **7.** Physa pomilia (ANSP 422687) **8.** *Physa carolinae*, new species (holotype, ANSP 422688).



Figures 9–10. The head regions of *Physa* species, bisected to reveal the penial complex in situ. **9.** *Physa acuta*. **10.** *Physa pomilia* and *P. carolinae* new species. Abbreviations: **bm,** buccal mass; **bmrmu,** buccal mass retractor muscles; **cg,** cerebral ganglion; **e,** eye; **f,** foot; **gprep,** preputial gland; **prep,** preputium; **rm,** reflected mantle; **sgl,** salivary gland; **spg,** glandular portion of penial sheath; **spm,** muscular portion of penial sheath.

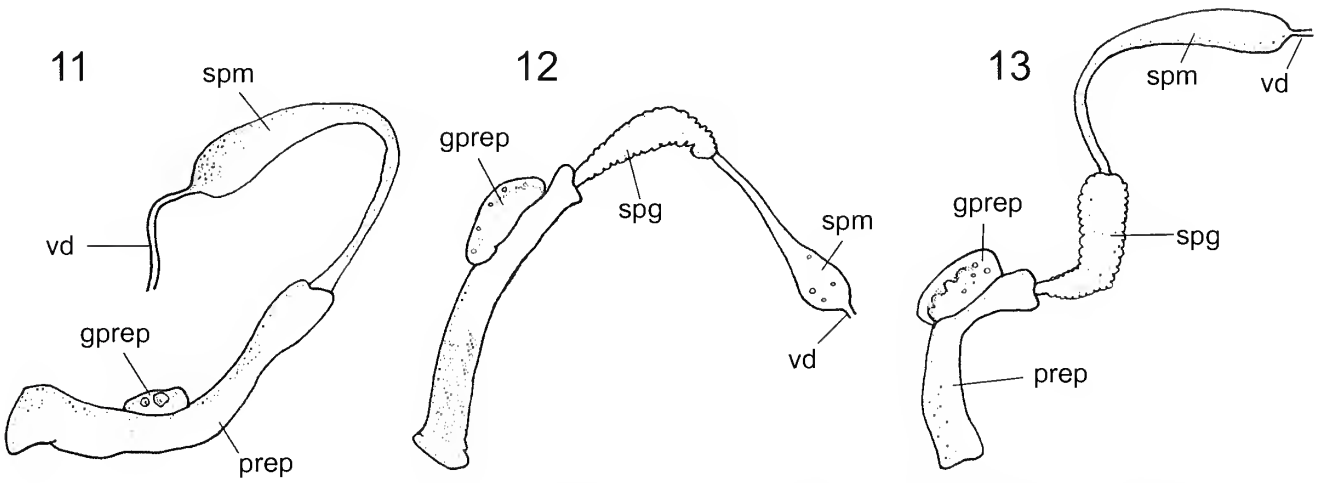
Physa pomilia hendersoni Clench, 1925a: 4, pl. 1, fig. 3.
Physa barberi Clench, 1925b: 2, pl. 1, fig. 1–3.
Physella hendersoni hendersoni Te, 1980: 184; Burch, 1989: 188, figs 675–677.
Physella hendersoni floridana "Pilsbry MS" Te, 1980: 184.

Description: The shell and anatomical morphology have not been well-characterized previously. They are similar in most respects to *Physa acuta*, with exceptions as noted below. Shell (Figure 7) sinistral, elongate-ovate, high spired, thin, translucent, lustrous, with faint spiral growth lines. Body whorl approximately 85% of shell length, with four to five adult whorls, with rounded shoulders but sutures not so deeply impressed as *P. acuta*. Spire profile flat to slightly convex, apex more rounded than *P. acuta*. Moderately auricular aperture, approximately 70% of shell length, with thin outer lip. Adulthood is reached quite rapidly in culture and at a small size. Dillon et al. (2007) reported a modal age of 4 weeks at first reproduction and Dillon (in review) recorded 7 weeks post-hatch. Growth rate seems to decrease markedly at maturity, such that individuals rarely attain shell lengths much greater than 7 mm. From the regression shown in Figure 5, the predicted length to width ratio would be 1.66 for a 6 mm animal and 1.79 for a (hypothetical) 8 mm animal. Cephalopedal mass (Figure 10) light gray to tan, with long, slender tentacles and rounded or fan-like labial palps. Jaw simple, lacking lateral processes. Mantle typically bearing a reticulate pigmentation pattern, sometimes demonstrating digitations. Foot extending approximately the length of the shell, pointed posteriorly. Penial complex (Figure 12) includes a preputium (with preputial gland) and a two-part penial sheath, which is divided into a muscular portion and a (smaller) glandular portion. This general penial morphology has been characterized as "type-bc" (Te, 1978; Wethington and Lydeard 2007). When everted, the penis slides through the preputium to form a long, slightly irregular, finger-like projection, with a lateral lobe corresponding to the preputial gland. Radula not different from *P. acuta* - comprising approximately 30–40 V-shaped rows of approximately 120–160 comb-like teeth. Each row has a tricuspid median flanked by 60–80 teeth bearing approximately 8–12 cusps.

Vouchers: Academy of Natural Sciences of Philadelphia, 20 dry shells (ANSP 422687) and 10 in 100% ethanol (ANSP A21950).

Type Locality: Randon's Creek, near Claiborne, Alabama.

Synonymy: Clench (1925) originally proposed *hendersoni* as a subspecies of *Physa pomilia*. Te (1978; 1980) reduced *pomilia* to subspecific rank under *P. heterostropha* (a junior synonym of *P. aeuta*), and elevated *hendersoni* to the full species level. The breeding experiments of Dillon et al. (2007) confirmed, however, that *P. hendersoni* is conspecific with *P. pomilia*, as originally suggested by Clench, and that populations of *hendersoni/pomilia* are reproductively isolated from *heterostropha/acuta*. These observations have been corroborated by DNA sequence data, which cluster *P. hendersoni* and *P. pomilia* in a monophyletic group separate and distinct



Figures 11–13. Extracted penial complexes of *Physa* species. **11.** *Physa acuta*. **12.** *Physa pomilia*. **13.** *Physa carolinae* new species Abbreviations: **gprep,** preputial gland; **prep,** preputium; **spg,** glandular portion of penial sheath; **spm,** muscular portion of penial sheath; **vd,** vas deferens.

from the larger group that includes *P. acuta* (Wethington, 2004; Wethington and Lydeard, 2007).

Distribution and Habitat: *Physa pomilia* appears to inhabit much of the eastern and southern United States, although confusion with *P. acuta* makes the actual extent of its range uncertain. In South Carolina, *P. pomilia* is moderately common in the slow pools and backwaters of rivers draining the coastal plain, typically on vegetation, both submerged and emergent. The water of such rivers is often colored with tannins, but probably not strongly acidic. *Physa pomilia* populations are not typically associated with polluted or otherwise disturbed habitats.

Physa carolinae new species
(Figures 1–13, 15)

Physa heterostropha, "JNI population."—Dillon and Wethington, 1995: 400–408.

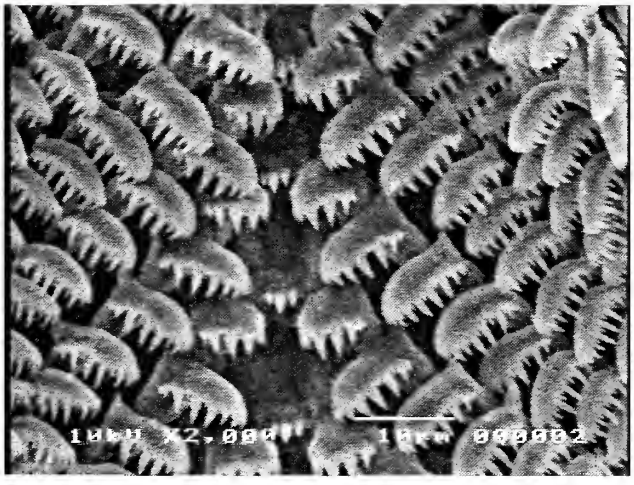


Figure 14. SEM microphotograph showing the radular morphology of *Physa acuta*.

Physa sp. "John's Island." —Wethington, 2004: 18–19.
Physa species A.—Wethington and Lydeard 2007: 241–257.
Physa species A.—Dillon (in review)

Description: The shell and anatomical morphology are similar in most respects to *Physa pomilia*, with exceptions as noted below. Shell (Figure 8) sinistral, narrowly elongate-ovate, high spired, thin, translucent, lustrous, with faint spiral growth lines. Body whorl approximately 85% of shell length, with four to five adult whorls, with rounded shoulders but sutures not deeply impressed. Spire profile flat to slightly convex, apex not acute. Moderately auricular aperture, approximately 70% of shell length, with thin outer lip. In culture, adulthood is reached at a modal age of 8 weeks post-hatch, approximately the same as in *P. acuta*, but at a later age and larger shell length than demonstrated by *P. pomilia* (Dillon, in review). From the regression shown in Figure 5, the predicted length to width ratio of a 6 mm animal would be 1.79, and for an 8 mm animal 1.92. Cephalopedal mass (Figure 10) generally black, much darker than *P. pomilia*, with long slender tentacles and rounded or fan-like labial palps. Jaw simple, lacking lateral processes. Mantle typically black, without reticulation, sometimes demonstrating digitations. Foot extending approximately the length of the shell, pointed posteriorly. Penial complex (Figure 13) including a preputium (with preputial gland) and a two-part penial sheath which is divided into a muscular portion and a (smaller) glandular portion. This general penial morphology has been characterized as "type-bc" (Te, 1978; Wethington and Lydeard 2007). When everted, the penis slides through the preputium to form a long, slightly irregular, fingerlike projection, with a lateral lobe corresponding to the preputial gland. Radula not different from *P. acuta*, comprising approximately 30–40 V-shaped rows of approximately 120–160 comb-like teeth. Each

row has a tricuspid median flanked by 60–80 teeth bearing approximately 8–12 cusps.

Type: The dry holotype has been deposited at the Academy of Natural Sciences of Philadelphia (ANSP 422688). We have also deposited 19 dry paratypes (ANSP 422689) and 10 paratypes in 100% ethanol (ANSP A21948).

Type Locality: Small spring at Huger Landing on the bank of Huger Creek, 4 km North of Huger, Berkeley County, South Carolina (33.1305°N, 79.8111°W). Springs are unusual in the South Carolina lowcountry, and this is the only population of *Physa carolinae* inhabiting such a habitat of which we are aware. We selected this type locality because the site is on public land, easily accessible, and snails can be sampled year round. Snails are also seasonally abundant in the ditch by the dirt road leading to the landing, which is a more typical habitat.

Distribution and Habitat: The natural habitat of *Physa carolinae* seems to be the broad and shallow waters of forested swamps in the lower coastal plain, such as Hellhole Bay in the Francis Marion National Forest or Wassamassaw Swamp west of Moncks Corner, SC. Such swamps typically swell with the rains of winter and spring and recede in the heat of summer. But because the thick base of spongy organic debris that builds up on the floor of such swamp forests never evaporates to dryness, snails are able to find refuge by burrowing. This life habit is similar to that displayed by the circumboreal physid genus *Aplexa*, which *P. carolinae* superficially resembles. The southern Atlantic Coastal plain has, however, been heavily impacted by human land use practices for several hundred years. *Physa carolinae* is today most commonly collected in manmade drainage ditches by roads and agricultural fields.

In addition to the type locality and the five supplementary populations sampled for DNA analysis, we have South Carolina records of *P. carolinae* as follows: Barnwell Co: Lower Three-Runs Ck 2 km W of Lyndhurst at S-39 (33.13°N, 81.45°W). Berkeley Co: Wassamassaw Swamp at US 176 (33.15°N, 80.17°W). Main pond at Cypress Gardens (33.0477°N, 79.9490°W). Charleston Co: Pond at Drayton Hall Plantation (32.8703°N; 80.0769°W). Ditch at Dill Wildlife Refuge, W of Riverland Dr., Charleston (32.7272°N; 79.9875°W). Reserve Pond, Santee Coastal Preserve, 10 km NE of McClellanville (33.1546°N, 79.3567°W). Jasper Co: Coosawhatchie Swamp, 2 km N of Coosawhatchie (32.6096°N, 80.9270°W).

The range of *P. carolinae* extends through the coastal plain and lower piedmont regions of Virginia, North Carolina, and Georgia (Figure 15). We have collections and observations on approximately 20 populations of *P. carolinae* in Virginia, 35 populations in North Carolina, and 20 populations in Georgia (available from RTD on request). Our extensive field surveys have not uncovered any populations inhabiting the upper piedmont or mountains to the west. We have no personal observations north of Virginia or south of Georgia. But the

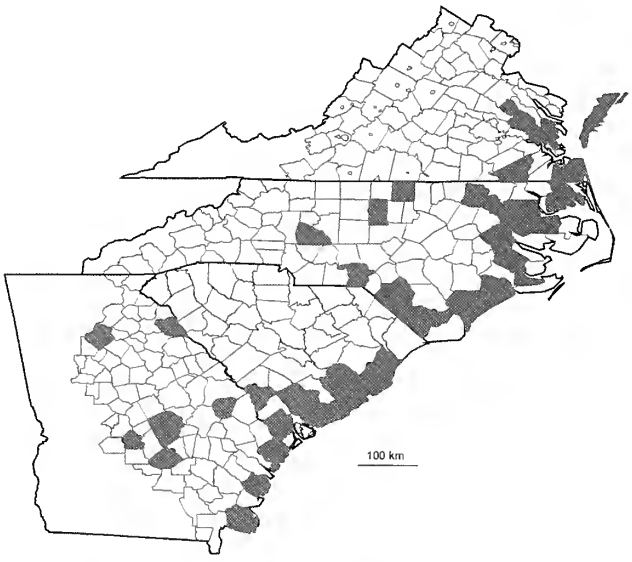


Figure 15. Counties in the southern Atlantic drainages of the United States with records of *Physa carolinae* new species.

collection of the Florida Museum of Natural History in Gainesville holds a large number of physid lots from Florida, catalogued primarily under the name "*Physa hendersoni*," that appear to represent *Physa carolinae*.

Etymology: Latin *carolinae*, genitive case of *carolina* meaning of Carolina (this species is first described from populations in South Carolina).

DISCUSSION

The genetic and morphological evidence reviewed in the present work, together with the experimental breeding results of Dillon (in review), make it clear that a widespread and seasonally common species of freshwater gastropod has escaped the attention of malacologists in the American South for almost two centuries. Part of the explanation doubtless lies in the difficult and ephemeral nature of its habitat. *Physa carolinae* populations are most often found in coastal plain swamps that are seasonally flooded and hence difficult to access, or in the ditches of disturbed habitats not typically surveyed by field biologists.

A second explanation for the protracted obscurity of *Physa carolinae* must be the longstanding confusion that has persisted in the taxonomy and systematics of the North American Physidae. The newly described species often lives in close proximity with two other earlier-described physid species which themselves have often been confused, *Physa acuta* (previously identified as *P. heterostropha*) and *Physa pomilia* (previously *P. heterostropha pomilia* or *P. hendersoni*). We ourselves misidentified a population of *P. carolinae* as *P. heterostropha* in our early surveys of allozyme variation among physids in the Charleston area (Dillon and Wethington, 1995). Once the previously described species were better

characterized and distinguished from each other (Dillon et al., 2007), the undescribed third species became easier to recognize.

We do not think that our experience with the physids of South Carolina will prove to be unique. Future studies combining genetic, morphological, ecological, and behavioral data will likely continue to prompt taxonomic revisions of even the most familiar elements of the North American freshwater gastropod fauna into the future.

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Spermatozoan ultrastructure and detection of nuclear acid phosphatase activity in spermatids of *Anomalocardia brasiliana* and *Tivela mactroides* (Bivalvia: Veneridae)

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ABSTRACT

We compared the ultrastructure of spermatozoa from the bivalves *Anomalocardia brasiliana* and *Tivela mactroides* (Veneridae). The spermatozoa of both species were of the *ect-aquasperm* type in which the head contains a curved nucleus with a short cone-shaped acrosome. An invagination penetrated almost the entire length of the acrosome. The midpiece contained a pair of orthogonally arranged centrioles surrounded by spherical mitochondria and the flagellum had the typical 9 + 2 structure. The spermatozoa of *A. brasiliana* had a slightly curved nucleus while those of *T. mactroides* had a long, prominently curved nucleus. The mitochondria were equally distributed around the centrioles in the midpiece of *A. brasiliana* spermatozoa, but asymmetrically in the midpiece of *T. mactroides* spermatozoa. There were six mitochondria and glycogen clusters in the middle piece of the *T. mactroides* spermatozoon. The presence of glycogen clusters and the higher number of mitochondria, in comparison with *Anomalocardia brasiliana*, could extend the longevity of the *Tivela mactroides* spermatozoa. An increase in sperm life expectancy implies in an increase in the probability of finding eggs and accomplishing fertilization. The glycogen clusters and the higher mitochondria number possibly correspond to an adaptive advantage to the bivalves in turbulent waters.

INTRODUCTION

Comparative studies of the Bivalvia have confirmed the usefulness of spermatozoan morphology for taxonomic and phylogenetic analyses (Bernard and Hodgson, 1985; Guerra et al., 1994; Sousa and Oliveira, 1994; Healy, 1995a, b; Garrido and Gallardo, 1996; Komaru and Konishi, 1996; Healy et al., 2001; Erkan and Sousa, 2002; Gwo et al., 2002; Introíni et al., 2004; Healy et al., 2006). In addition, these studies showed that closely related species can be distinguished based on the ultrastructure of their spermatozoa (Hodgson et al., 1990; Gwo et al., 2002; Introíni et al., 2004).

Various features of spermatozoan ultrastructure have been associated to aspects of reproductive biology. Franzén (1955, 1956, 1977, 1983) proposed that invertebrates have two types of sperm, namely, *primitive* sperm, produced by species with external fertilization, and *modified* sperm, produced by species with internal fertilization. Primitive sperm consists of a short, round or conical head, a midpiece containing 4–5 spherical mitochondria and a flagellum with a 9+2 microtubular structure. Spermatozoa that are released directly into the surrounding water are named *aquasperm* or *aquatic sperm*. Rouse and Jamieson (1987) introduced a new terminology and described two *aquasperm* categories: (1) *ect-aquasperm* referring to sperm that fertilizes eggs in the ambient water; (2) *ent-aquasperm* referring to sperm that fertilizes eggs into the mantle cavity of molluscs or into the tube of sedentary polychaetes.

Sperm morphology has also been correlated with egg size and larval development (Franzén, 1983; Komaru and Konishi, 1996). These relationships among spermatozoan morphology and distinct reproductive patterns have strengthened the relevance of studies that investigate

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the association of evolution, ecology, and the morphological diversity of spermatozoa.

The Veneroida is considered one of the most important orders of bivalves because it comprises several marine families of economic, ecological relevance and widespread geographic distribution, such as the Veneridae (Gwo et al., 2002). Data describing spermatozoan ultrastructure of Veneridae species have supported the identification of traits shared by the majority of the members belonging to this family (Pochon-Masson and Gharagozlou, 1970; Gharagozlou and Pochon-Masson, 1971; Nicotra and Zappata, 1991; Reunov and Hodgson, 1994; Guerra et al., 1994; Matos et al., 1997; Gwo et al., 2002; Erkan and Sousa, 2002; Park et al., 2002; Guerra et al., 2003; Ying et al., 2008).

Numerous reports have emphasized that venerid bivalves are important components of the marine benthos, including non-consolidated bottom communities (Narchi, 1972; Etchevers, 1976; Schaeffer-Novelli, 1980; Prieto, 1980; Soares et al., 1982; Prieto, 1983; Mclachlan et al., 1996; Arruda and Amaral, 2003; Arruda et al., 2003). *Anomalocardia brasiliana* (Gmelin, 1791) is distributed throughout the Caribbean islands and also in Suriname, Brazil and Uruguay (Amaral et al., 2005). This species lives buried a few centimeters below the surface of compact sand in the intertidal zone of calm waters. Adults of *A. brasiliana* rapidly bury themselves when placed on wet mud or muddy sand. *Tivela mactroides* (Born, 1778) occurs around the Ascension Island, along the Caribbean seaboard, and in Venezuela, Suriname and Brazil (Amaral et al., 2005). In contrast to *A. brasiliana*, *T. mactroides* lives in turbulent waters in the intertidal zone.

Although, the spermatozoa ultrastructure of bivalves has been largely investigated and used to solve many taxonomic and phylogenetic issues, immune and cytochemical studies of sperm and precursory cells are scant. Biochemical features of reproductive cell lineages could contribute to taxonomical descriptions and to distinguish some of the bivalve species.

In this work, scanning and transmission electron microscopy were used in the study of the spermatozoan morphology of the venerids *Anomalocardia brasiliana* and *Tivela mactroides*, which were compared with those of other bivalve species, aiming at a better understanding of their taxonomic placement and phylogeny.

MATERIALS AND METHODS

Specimens of *Anomalocardia brasiliana* were sampled in the intertidal zone of São Sebastião County (23°48′57.2″ S, 45°24′29.8″ W), and *Tivela mactroides* sampled in Caraguatatuba County (23°38′51.7″, 45°25′31.4″ W), both located on the southeastern coast of São Paulo State, Brazil.

The voucher specimens were deposited in the Museu de Zoologia "Professor Adão José Cardoso" (ZUEC) at the State University of Campinas (UNICAMP), São Paulo, Brazil, under the accession numbers 1419 (Tivela mactroides) and 1420 (*Anomalocardia brasiliana*).

Scanning Electron Microscopy: Sperm suspensions on coverslips were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.2 M sodium cacodylate, pH 7.2, for 1 h at room temperature. Subsequently, they were rinsed several times in the same buffer and post-fixed in 2% osmium tetroxide in the dark for 1 h. Samples were dehydrated in a graded series of ethanol solutions and critical-point dried in CO2. The dried coverslips were mounted on stubs, coated with gold and examined with a JSM 5800 LV microscope.

Transmission Electron Microscopy: Small fragments of testis were fixed with 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.2 M sodium cacodylate, pH 7.2, for 5 h at 4°C and then rinsed in the same buffer. Samples were post-fixed in 2% osmium tetroxide in the same buffer, for 1 h at 4°C, and then dehydrated in a graded acetone series followed by gradual infiltration with EPON resin before embedding. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss Leo 906 transmission electron microscope.

Phosphotungstic Acid Staining: Small samples of testis were fixed as described above, but without post-fixation, and subsequently dehydrated in a graded ethanol series. Samples were stained with a 2% phosphotungstic acid-ethanol (E-PTA) solution at low pH, in order to detect glycoproteins and enable cytochemical analysis of spermatozoa. After 2 h in the E-PTA solution, samples were rinsed with ethanol, transferred to acetone, and infiltrated with EPON resin before embedding. Ultrathin sections were examined with a Zeiss Leo 906 transmission electron microscope.

Nuclear ACPase Detection During Spermiogenesis: Small fragments of testis were fixed with 2.5% glutaraldehyde in 0.2 M sodium cacodylate, pH 7.4, for 2 h at 4°C, and rinsed in the same buffer. They were then washed with 50mM Na-acetate-HCl buffer, pH 5, at 4 °C. Subsequently, the testis fragments were incubated in 50mM Na-acetate-HCl buffer, pH 5, with 5% sucrose, 13.9 mM sodium β-glycerophosphate (β-GP) and 3.6 mM lead nitrate, for 30 min at 37°C, under dark conditions with constant and gentle mixing. Samples were washed twice in 50mM Na-acetate-HCl buffer, pH 5, with 5% sucrose, for 5 min at 4°C, and twice in 0.1 M Na-cacodylate buffer, pH 7.2, with 5% sucrose, for 5 min at 4°C. The specimens were post-fixed in 2% osmium tetroxide in the same buffer, for 2 h at 4°C, and then dehydrated in a graded ethanol series and propylene oxide. A gradual infiltration with EPON resin was done before embedding. Ultrathin sections were examined with a JEOL 100 CX II-TEM, without staining.

The experimental controls consisted of: (1) Omission of β-glycerophosphate; (2) Addition of inhibitor 10mM NaF; (3) Other substrates: 6.4 mM Thiamine pyro-

phosphate chloride; 2.5 mM Na-inosine- 5-diphosphate; 0.6 mM Na-trimetaphosphate.

RESULTS

Spermatozoa: The spermatozoa of both species were either of the *cct-aquasperm* type (Figures 3, 4, and 12). The short acrosomal complex was cone-shaped and located anterior to the nucleus. Two components of the acrosomal vesicle were distinguished based on their diverse electron densities (Figures 20 and 22). The conical acrosome was deeply invaginated, the subacrosomal region was filled with a diffuse material, and there was no axial rod (Figures 1, 2, 10, 11, and 13).

PTA staining at low pH revealed no glycoproteins in the acrosomal vesicles of *Anomalocardia brasiliana* and *Tivela mactroides* spermatozoa (Figures 5, 6 and 14). The nucleus was relatively long, curved, cylinder, and the midpiece consisted of spherical mitochondria grouped around a pair of short cylindrical centrioles (Figures 7, 8, 15, 16, and 17). Extensive electron-dense granules or granule clusters, considered to be glycogen deposits, were observed around the centrioles and mitochondria of *T. mactroides* spermatozoa (Figures 15 and 16). In the region immediately posterior to the midpiece, the triplet substructure of the centrioles was replaced by a standard 9 + 2 microtubular pattern axoneme that terminated in a long flagellum (Figures 9 and 18). Overall, the spermatozoa of *A. brasiliana* and *T. mactroides* shared high morphological similarities, even though the nucleus was slightly curved in the *A. brasiliana* spermatozoa compared to the markedly curved and long nucleus of *T. mactroides* (Table 1, Figures 19 and 24). The mitochondria were equally distributed around the centrioles in the midpiece of *A. brasiliana* spermatozoa (Figure 21). In *Tivela mactroides* spermatozoa, the mitochondrial assembly did not form a ring structure but showed a biased distribution of the organelles around the orthogonally arranged pair of centrioles, such that they were always more numerous in one side of the sperm cell (Figure 23). Hence, the midpiece of *T. mactroides* spermatozoa was rotationally asymmetrical and contained clusters of glycogen, which were not seen in the midpiece of *A. brasiliana*.

Precursory Cells: The nomenclature used to refer to precursory cells was that of Nicotra and Zapata (1991), which is not in accordance with the definitions proposed by Ying et al. (2008).

The early spermatids were rounded but irregular in outline and had a spherical nucleus with patches of condensed chromatin in the middle and in the cell periphery (Figures 25 and 29). The nucleus was still rounded in the mid-spermatid stage, but the chromatin condensation was intensified (Figures 26 and 30).

The late spermatid was characterized by the elongation of the nucleus. Chromatin condensation has completed and only a few nuclear vacuoles remained. While the residual cytoplasm was progressively eliminated, spherical mitochondria assembled in the base of the nucleus around the two centrioles (Figures 27 and 31).

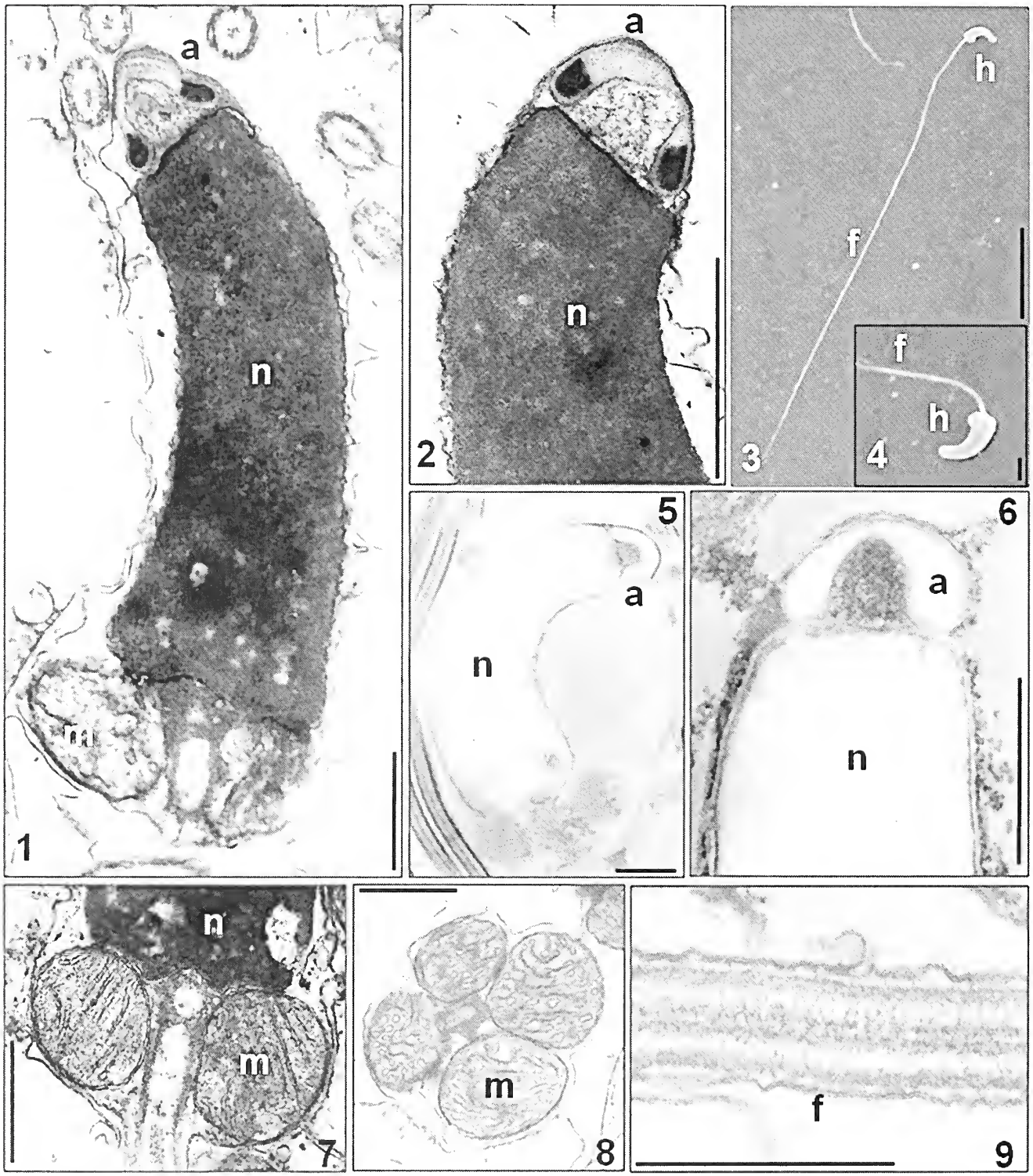
In *Anomalocardia brasiliana* gonads, a nuclear acid phosphatase (ACPase) was detected in all spermatid stages using the improved Gomori-chloride technique. In comparison, in *Tivela mactroides* gonads, the presence of ACPase was detected in mid and (inconspicuously) late spermatids using the same methodology. In the sperm cell of both species, nuclear ACPase was not detected (Figures 28 and 32). The experimental controls did not show staining.

DISCUSSION

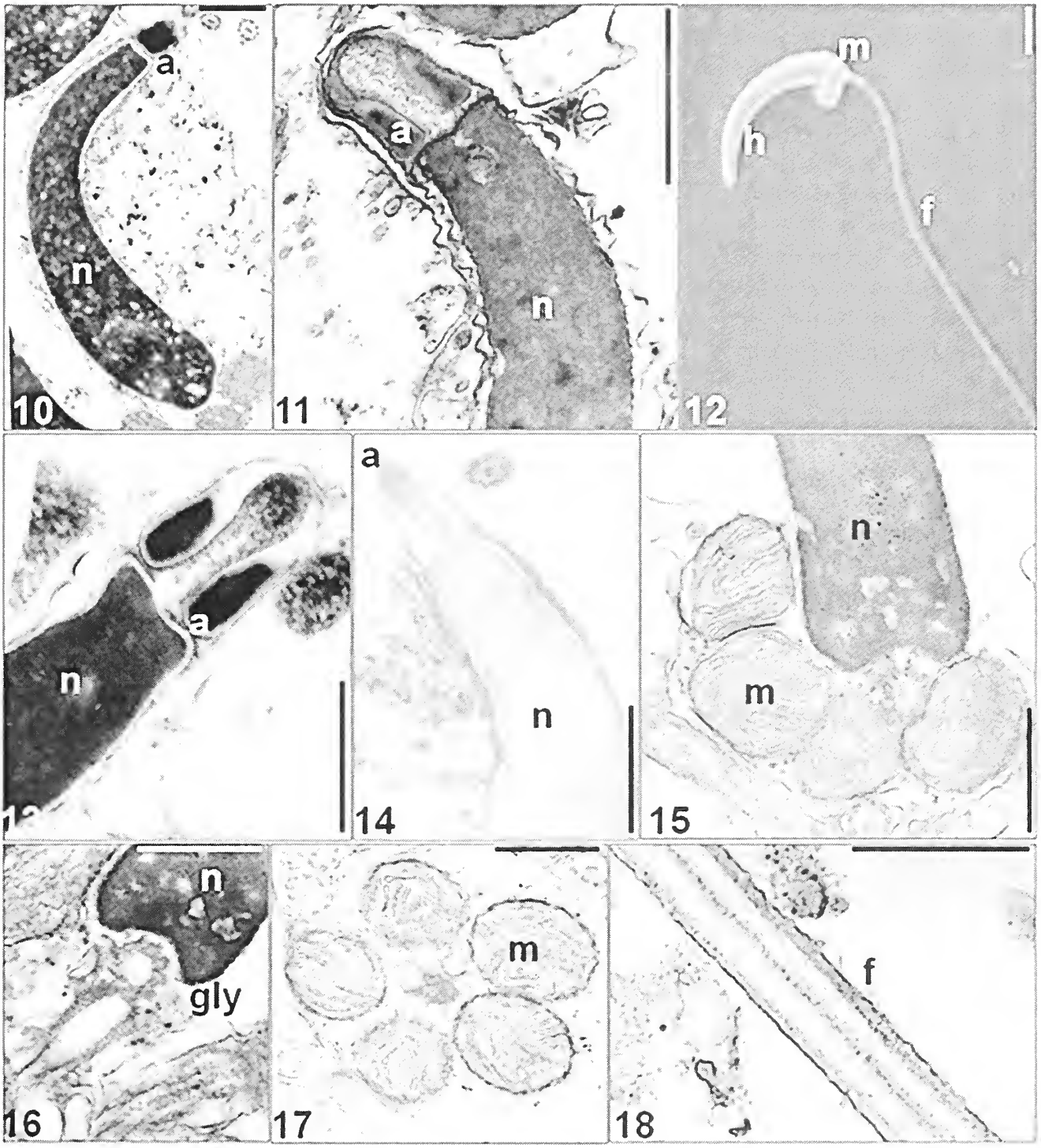
The morphological structures of the venerid spermatozoa described herein agree with the *cct-aquasperm* type proposed by Rouse and Jamieson (1987), exhibiting morphological characteristics described for free-spawning bivalves. In a previous study of bivalve sperm ultrastructure, Healy et al. (1995b) proposed five categories within the order Veneroida. The spermatozoa descriptions presented here are in agreement with the data reported by Healy et al. (1995b) regarding members belonging to Group A. Members of Group A share the following traits: a randomly organized subacrosomal material, an electron-lucent area at the acrosomal apex, a relatively long and slender rod nucleus that slightly decreases in thickness toward the gamete apex, and absence of an anterior nuclear fossa.

As described below, previous studies described detailed ultrastructural patterns of venerid spermatozoa; the reported data allows for an informal taxonomic analysis of this bivalve group.

Venerupis aurea (Gmelin, 1791) produces a spermatozoon with a very slightly curved nucleus and electron-dense regions at the base of the acrosomal vesicle (Gharagozlou and Pochon-Masson, 1971). Nicotra and Zappata (1991) investigated the sperm cells of *Callista chione* (Linnaeus, 1758), which also exhibit nuclear curvature and the same pattern of acrosomal electron density. Reunov and Hodgson (1994) described the spermatozoan morphology of the venerid clam *Tivela polita* (G.B. Sowerby II, 1851) from South Africa. The head of *T. polita* spermatozoa is about 3.2 µm long and has a cylindrical, slightly curved nucleus capped by a small conical acrosome. In the venerid clams *Protothaca thaca* (Molina, 1782) and *Ameghinomya antiqua* (King and Broderip, 1832) (as *Venus antiqua*), the reported length of the sperm head is about 7.5 µm and 5.3 µm, respectively, and the acrosome is a small vesicle in the anterior region of the cell (Guerra et al., 1994). Sperm ultrastructure studies of *Protothaca pectorina* (Lamarck, 1818) from the northern coast of Brazil showed that the male gamete exhibits a curved nucleus (Matos et al., 1997). The species *Gafrarium tumidum* Röding, 1798, and *Circe scripta* (Linnaeus, 1758) (Circinae), *Pitar sulfureum* Pilsbry, 1804 (Pitarinae), and *Gomphina aequilatera* (G.B. Sowerby I, 1825) (Tapetinae) share highly similar spermatozoa



Figures 1–9. Spermatozoon of *Anomalocardia brasiliana*. **1.** Acrosome, nucleus and midpiece. **2.** Acrosomal complex and nuclear apex. **3–4.** SEM showing head and flagellum. **5–6.** Phosphotungstic acid staining. **7.** Longitudinal section of the midpiece. **8.** Transversal section of the midpiece showing four spherical mitochondria grouped as a ring around the proximal centriole. **9.** Longitudinal section of the flagellum. Scale bars = 0.5 µm, except for Figure 3 = 10 µm and Figure 4 = 1 µm. Abbreviations: **a,** acrosome; **f,** flagellum; **h,** head; **m,** mitochondria; **n,** nucleus.



Figures 10–18. Spermatozoon of *Tivela mactroides*. **10.** Acrosome, nucleus and midpiece. **11.** Acrosomal complex and nuclear apex. **12.** SEM showing head and flagellum. **13.** Acrosomal complex and nuclear apex. **14.** Lack of phosphotungstic acid staining in the acrosomal vesicle. **15.** Longitudinal section of the midpiece, showing six spherical mitochondria grouped around the proximal centriole. **16.** Longitudinal section of the midpiece. **17.** Spherical mitochondria grouped around the distal centriole. **18.** Longitudinal section of the flagellum. Scale bars = 0.5 µm, except for Figure 12 = 1 µm. Abbreviations: **a,** acrosome; **f,** flagellum; **gly,** glycogen clusters; **h,** head; **m,** mitochondria; **n,** nucleus.

Table 1. Morphometric and numerical data of analyzed sperm structures in *A. brasiliana* and *T. mactroides*.

Species	Acrosomal length (μm)	Nuclear width (μm)	Head length (μm)	Number of mitochondria
A. brasiliana	0.4	0.9	3	4
T. mactroides	0.4	0.5	4.5	5–6

morphology and ultrastructure, as reported by Gwo et al. (2002). Sperm cells of these bivalve species consisted of a curved head, a short midpiece with tightly packed mitochondria and a long tail. The spermatozoa of the bivalve mollusks *Pitar rudis* (Poli, 1795) and *Chamelea gallina* (Linnaeus, 1758) (Veneridae) from Turkey were characterized by a conical and slightly curved nucleus about 2.6 μm and 3.5 μm long, respectively, and acrosomal vesicles about 0.6 μm long (Erkan and Sousa, 2002). The sperm cell of *Gomphina veneriformis* (Lamarck, 1818) exhibited a conspicuous curvature and the head (8.5 μm) is considered very long (Park et al., 2002). Sperm cells of the clam *Mercenaria mercenaria* (Linnaeus, 1758) (from China), were investigated by Ying et al. (2008), sharing common features with other venerid species, such as the presence of a curved nucleus and a short acrosomal vesicle which shows electron dense regions in its base.

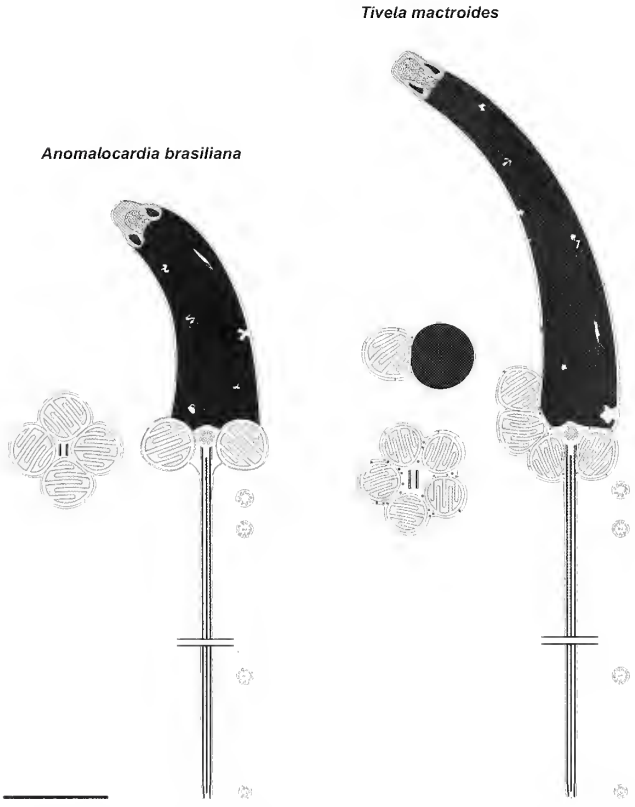


Figure 19. Diagrammatic representation of *A. brasiliana* and *T. mactroides* spermatozoa. Scale bar = 1 μm.

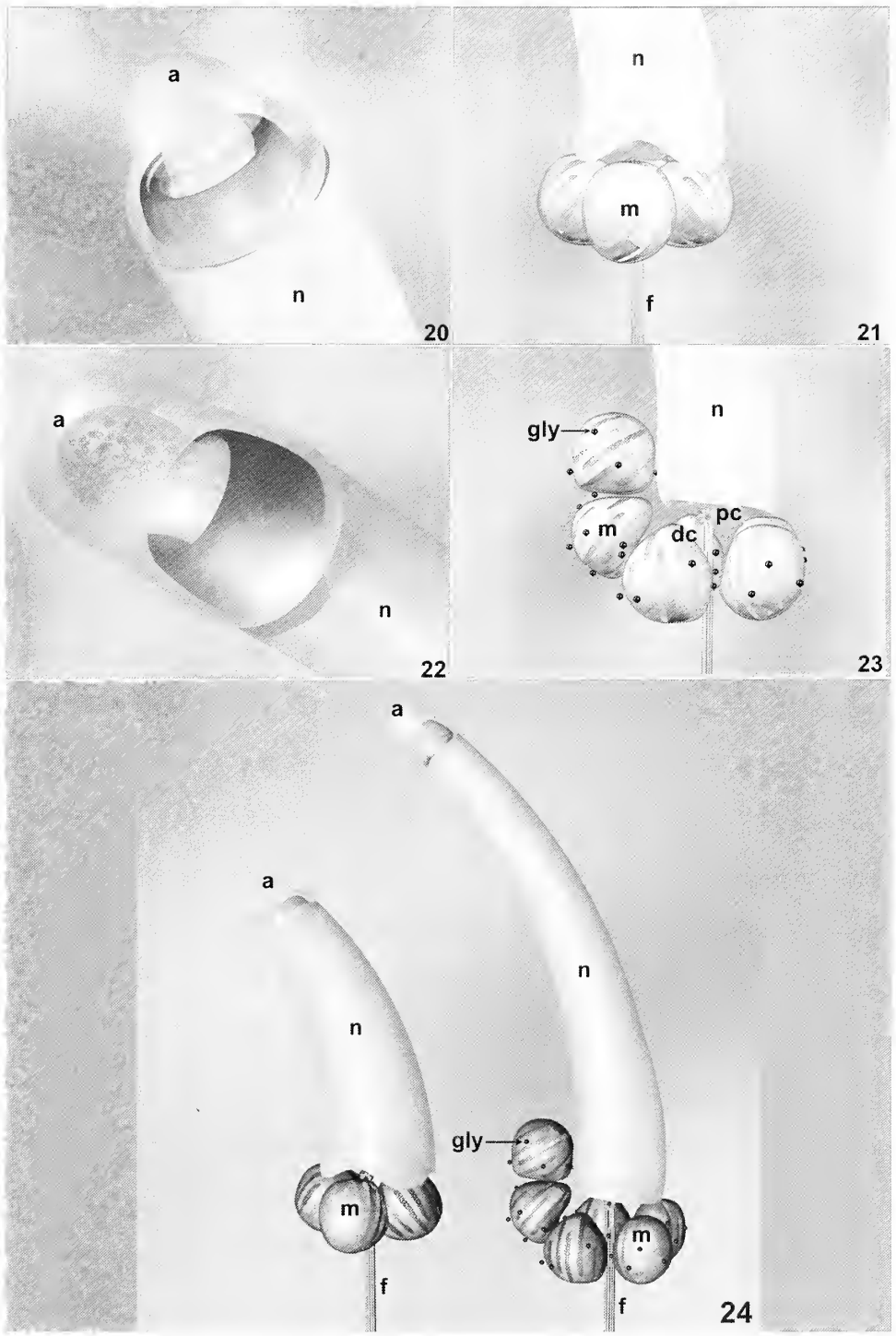
The elongated head of venerid spermatozoa usually contains a curved nucleus, which characterizes these bivalves (Reunov and Hodgson, 1994). However, the nucleus curvature is not unique to venerids since it has also been reported in galeommatoidean bivalves (Eckelbarger et al., 1990). Nicotra and Zappata (1991) stated that it should be interesting to analyze movement and fertilization patterns in this curved sperm, in order to evaluate the functional significance of this characteristic. However, considering the microscopic dimensions of the sperm cells, the nucleus shape should be irrelevant to the movement because these bodies have low Reynolds numbers, not being possible to derive any appreciable thrust from inertia.

The apical half of the spermatozoon acrosomal vesicle is stained with PTA in the species *Ruditapes decussatus* (Linnaeus, 1758) (as *Venerupis decussatus*), *Eurhomalea rufa* (Lamarck, 1818), *Protothaca thaca,* and *Venus antiqua* (family Veneridae), as reported by Sousa et al. (1998). This is in contrast to the negative PTA staining pattern of *Anomalocardia brasiliana* and *Tivela mactroides* spermatozoa.

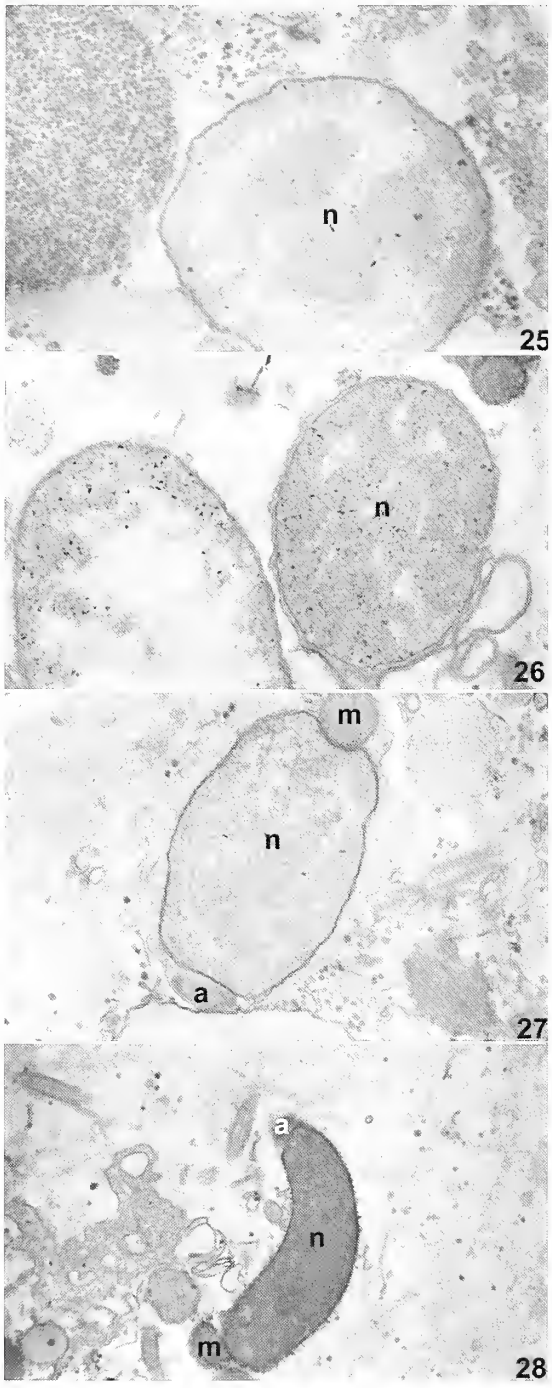
Sperm morphological studies described non-curved nucleus in the venerid *Eurhomalea rufa* (Sousa et al., 1998, Guerra et al., 2003). The nucleus of sperm cells were curved in all other studied venerid species. The ultrastructural characteristics of *Ruditapes decussatus* (Pochon-Masson and Gharagozlou, 1970; Gharagozlou and Pochon-Masson, 1971) were different in comparison to other venerid species, especially in relation to acrosomal features. It is important to emphasize that the sperm cell of *Eurhomalea rufa* shows significant morphological differences in comparison with other species of the family Veneridae. These sperm cells differences have not yet been explained, representing an invitation to a taxonomic review of these species.

Besides their similarities, *A. brasiliana* and *T. mactroides* spermatozoa showed prominent ultrastructural differences. The sperm nucleus was slightly curved in *A. brasiliana* and prominently curved and long in *T. mactroides*. In the *A. brasiliana* sperm cell midpiece, there were four mitochondria uniformly distributed around the centrioles. As for *T. mactroides*, there were six asymmetrically distributed mitochondria. Finally, the midpiece of *T. mactroides* contained glycogen deposits whereas that of *A. brasiliana* did not. These morphological patterns suggest that the *T. mactroides* spermatozoa could be adapted to turbulent environments.

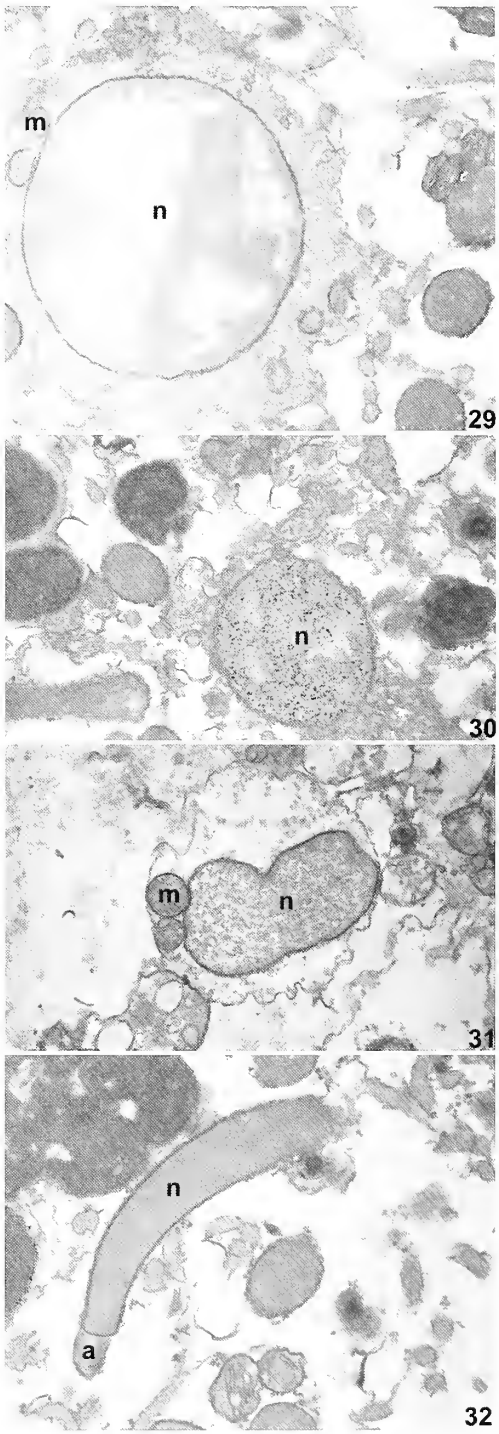
Narchi (1972) compared structural and functional morphologies as well as adaptations of *A. brasiliana* and *T. mactroides*, which are species living close to the surface in soft substrata with suspension-feeding habits. Their most prominent anatomical features were related to burrowing behavior and suspension-feeding. *Anomalocardia brasiliana*, which lives in calm waters of muddy beaches, does not have tentacles along the mantle edge whereas the inhalant siphon and mantle edge of *T. mactroides* have several ramified tentacles. The siphonal tentacles prevent the penetration of large particles into the mantle cavity while the ramified tentacles along the



Figures 20–24. Illustrations of spermatozoan features of *Anomalocardia brasiliana* and *Tivela mactroides* based on SEM and TEM images processed through 3D animation rendering and modeling software. **20.** Apex of the head of the spermatozoon of *A. brasiliana*. Acrosomal length = 0.4 µm. **21.** Middle piece of the spermatozoon of *A. brasiliana*. Nuclear width = 0.9 µm; mitochondrion = 0.55 µm. **22.** Apex of the head of the spermatozoon of *T. mactroides*. Acrosomal length = 0.5 µm. **23.** Middle piece of the spermatozoon of *T. mactroides*. Mitochondrion = 0.5 µm. **24.** General visualization of the spermatozoan features of both spermatozoa. Mitochondrion = 0.55 µm (in spermatozoan representation at right side of the figure.) Abbreviations: **a,** acrosome; **dc,** distal centriole; **f,** flagellum; **gly,** glycogen clusters; **h,** head; **m,** mitochondria; **n,** nucleus, **pc,** proximal centriole.



Figures 25–28. Precursory cells of *Anomalocardia brasiliana*. **25.** Early spermatids with spherical nucleus with patches of condensed chromatin in the middle and in the cell periphery. Note staining indicating the presence of nuclear ACPase. Nuclear diameter = 4.2 µm. **26.** Nucleus still rounded in mid-spermatid stage, but chromatin condensation was intensified (presence of nuclear ACPase). Nuclear width = 3 µm and nuclear length = 4 µm. **27.** Late spermatid characterized by elongation of nucleus (presence of nuclear ACPase). Mitochondrion = 0.6 µm. **28.** Nuclear ACPase not detected in the sperm cell. Mitochondrion = 0.6 µm. Abbreviations: **a,** acrosome; **m,** mitochondria; **n,** nucleus.



Figures 29–32. Precursory cells of *Tivela mactroides*. **29.** Early spermatids with spherical nucleus with patches of condensed chromatin in the middle and in the cell periphery. Mitochondrion = 0.6 µm. **30.** Nucleus was still rounded in mid-spermatid stage, but chromatin condensation was intensified (presence of nuclear ACPase). Nuclear diameter = 2.25 µm. **31.** Late spermatid characterized by the elongation of the nucleus. Mitochondrion = 0.6 µm. **32.** Nuclear ACPase not detected in the sperm cell. Mitochondrion = 0.6 µm. Abbreviations: **a,** acrosome; **m,** mitochondria; **n,** nucleus.

mantle edge prevent large particles from entering the pallial cavity. These adaptations allow *T. mactroides* to live on open sandy shores where large quantities of material are kept in suspension by constant wave movements. *Tivela maetroides* also has the most efficient form of particle transport among demibranchs, which partly reflects adaptation to a specific habitat. In agreement with this characteristic, the stomach of *T. maetroides* is more complex than that of *A. brasiliana*, which is related to the large number of particles present in this organ. Our observations on the spermatozoan morphology of *A. brasiliana* and *T. mactroides* mostly agree with Narchi (1972), who concluded that anatomical variations in these species reflect adaptations to diverse environments. According to Anderson and Personne (1970; 1976) and Introíni et al. (2009), glycogen storage in the middle piece of bivalve sperm cells has an important meaning in the spermatozoon physiological metabolism. The presence of glycogen clusters in the mid-piece could extend the longevity of the sperm cells. Any increase in the life expectancy of sperm cells implies in an increase in the probability of finding eggs and increase in opportunities for fertilization. This could be an adaptive advantage in turbulent waters.

Spermiogenesis in Veneroida species has been described with great accuracy emphasizing architectural details of cells (Nicotra and Zapata, 1991; Johnson et al., 1996, Ying et al., 2008). In the present work, the relatively low fixation shown in the electron micrographs of precursory cells was carried out intentionally, in order to avoid masking detection of Nuclear ACPase activity.

Considerable ultrastructural modifications take place during spermiogenesis. The chromatin filaments aggregate into lamellar structures and finally into a homogeneous and compact DNA arrangement. In *Anomalocardia brasiliana* and *Tivela mactroides* gonads, a nuclear acid phosphatase (ACPase) was detected in spermatids using the improved Gomori-chloride technique. In the present analysis, the nuclear acid phosphatase activity in both bivalve species follows a specific time and spatial-course pattern during spermatid chromatin condensation. A controlled and comparative study suggests that this pattern of nuclear acid phosphatase activity is specific and related to chromatin compaction.

In conclusion, our results suggest that detailed analyses of bivalve spermatozoan ultrastructure can be useful tools in the investigation of interspecific taxonomic relatedness and adaptation to a given environment. Further studies on male gametes of venerid mollusks are needed to verify the taxonomic relevance of sperm morphological and ultrastructural characteristics.

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Revising α-taxonomy in shelled gastropods: the case of *Rissoa panhormensis* Verduin, 1985 (Caenogastropoda: Rissoidae)

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ABSTRACT

In one of his landmark papers on the genus *Rissoa* (Caenogastropoda: Rissoidae), Verduin described the new species *Rissoa panhormensis*, based on a few empty shells. After its description, no new data on the species have been published and its taxonomic status has remained questionable. We studied the type material of *R. panhormensis* and several living specimens whose shells resembled those of *R. panhormensis*. Although we could differentiate *R. panhormensis* from *R. guerinii* on the basis of qualitative visual observations and geometric morphometric data, it was not possible to separate both taxa using body colour patterns and 16S + COI mitochondrial DNA sequence data. We therefore suggest that *R. panhormensis* may be a rare morphotype of *R. guerinii* and should be synonymized with this latter species.

Additional keywords: Gastropoda, mitochondrial DNA, geometric morphometry, variation, morphotype

INTRODUCTION

Most older gastropod species descriptions were limited to shell diagnoses, which for a long time were considered to be sufficient to justify specific taxa. However, more recent molecular systematic tools (e.g. Knowlton, 2000; Bickford et al., 2007) have often been used to invalidate species described purely on shell characters alone. On the other hand, cases in which genetic differentiation is hidden by similarity in shell morphology are not uncommon (e.g. references in Knowlton, 1993, 2000). Thus, the degree of shell morphological differentiation may not reflect genetic or anatomical differentiation even among congeneric species.

A purely conchological approach was followed by Verduin (1976, 1982, 1983, 1985, 1986) in his revision of the European species belonging to six subgenera of the genus *Rissoa* (Fréminville ms) Desmarest, 1814 (Gastropoda: Rissoidae). He took into account quantitative (shell measurements, number of sculptural elements) and qualitative shell features (colour pattern elements), assigning to the latter numerical values based on presence/absence or degree of intensity. For the species belonging to the subgenera *Loxostoma* Bivona-Bernardi, 1838, and *Rissoa* Verduin (1983, 1986) only provided narrative descriptions, but for the subgenera *Turboella* (Leach ms) Gray, 1847, *Rissostomia* Sars, 1878, *Goniostoma* (Megerle ms) Villa, 1841, and *Apicularia* Monterosato, 1884, Verduin (1976, 1982, 1985) presented more elaborate data. The shell characters examined in each paper were the same, except for some variation according to the main features of the group studied. In his papers, Verduin (1976, 1982, 1983, 1985, 1986) provided meticulous measurements which were summarized in classical representations (scatterplots or histograms) to facilitate the species comparisons. Verduin placed considerable importance on the dimensions of the shell apex. He observed that there was a larger and smaller type of apex, with no intermediates. According to Verduin (1986), shells sharing the same morphological features, but belonging to two different groups according to apex dimensions, must be considered members of separate species. This statement was supported by the observation that, in some *Rissoa* species (e.g. Rehfeldt, 1968), different types of apex are linked to different larval developmental strategy. A small apex is considered typical of a planktotrophic veliger and large apex is thought to be linked to a lecithotrophic larva (e.g. Thorson, 1950). It is commonly accepted for gastropods that there is no evidence of intraspecific polymorphism in developmental strategy (Bouchet, 1989). Verduin (1986) listed eight "pairs" of sibling species of *Rissoa* (*sensu* Mayr, 1963) that differed mainly in apex dimensions. In doing so, Verduin (1985) not only revised existing *Rissoa* species, but also described some as new. In his paper on the subgenera *Apicularia* and *Goniostoma* (Verduin, 1985),

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one of the new species, *R. panhormensis*, was based on seven empty shells in the Philippe Dautzenberg collection (now housed in the Royal Belgian Institute of Natural Sciences, RBINS).

Rissoa panhormensis, aecording to its original description (Verduin, 1985) was differentiated as follows: "The shells of *R. panhormensis* strongly recall the normal colour variety of *R. guerinii* (Dautzenberg and Durouchoux, 1914), but have the larger type of apex, i.e. $0.265 < D_0 + 0.72d < 0.290$ mm, and have only 5–6 ½ terminal ribs per whorl. The shells measure from 4.8 to 6.0 mm. All have punctate spiral striae on the lower part of the body whorl. For the remainder, the shells are covered with fine, dense spiral striae, which merge into the punctate spiral striae. There are 7.6–8.2 whorls and 3½–4¾ ribbed whorls. The ribs continue up to the labial rib. The labial rib is well developed, and of a whitish colour, as are the other ribs. As in the normal colour variety of *R. guerinii*, the uppermost whorls are of a remarkable greyish colour. The edge of the aperture is purplish." The type locality is Palermo (Sicily, Thyrrenian Sea).

Together with the description, Verduin (1985) provided a black and white photograph of the holotype, which until now is the only known picture of *Rissoa panhormensis*. After its description, the species slipped into obscurity with hardly a mention in the literature. Currently, *Rissoa panhormensis* is considered to be an endemic species of the Western-Central Mediterranean (Bodon et al., 1995). We examined the type material of *R. panhormensis* and other specimens of *Rissoa* in the Dautzenberg collection (RBINS). On the basis of that material we were able to critically revise the original description of the taxon and to verify shell measurements.

We also sampled living *Rissoa* spp., collecting some specimens whose shells closely resembled the description of *R. panhormensis*. This allowed us to study other features such as external soft body parts and mitochondrial DNA. Several empty shells corresponding to *R. panhormensis* were also found washed ashore. Based on this material we provide a morphological and molecular re-evaluation of the taxonomic status of *R. panhormensis*.

MATERIALS AND METHODS

Dautzenberg Collection Material: The type material of *Rissoa panhormensis* in the Dautzenberg collection comprises the holotype and six paratypes from Palermo (Sicily, Tyrrhenian Sea). Four other lots were selected, which, according to present taxonomy belonged to *R. guerinii* Recluz, 1843. The specimens of these latter lots closely resembled *R. panhormensis* type material. Table 1 summarises the data of the four lots and the acronyms used in this study to identify them. All the shells of the five lots were examined using an Olympus® SZX10 stereoscopic microscope. Damaged shells and misclassified specimens (e.g. *R. violacea* Desmarest, 1814) were not further considered. Only five shells from lot Rpt were considered. These five shells of *R. panhormensis* and 20 randomly chosen shells of *R. guerinii* (five from each of the four lots, Rca, Rcb, Rcm and Rsm), were selected for this study. The presence of a well-formed labial rib in all the shells indicated that they were all at terminal growth (Warén, 1996).

Table 1. The composition of the studied lots of *Rissoa* spp., with the inscription of the original label and their acronym. No further data (exact locality, date, etc.) regarding these lots were available.

Acronym	Content	Label
Rpt	6 *R. panhormensis* 1 *R. violaeea*	*Rissoa panhormensis* Verduin/Det.: Verduin, 1983
Rca	51 *R. guerinii* 2 *R. violaeea*	*Rissoa eostulata*
Rcb	92 *R. guerinii*	*Rissoa eostulata*, Alder
Rcm	15 *R. guerinii*	*Rissoa eostulata*, Medit. Monts.
Rsm	12 *R. guerinii*	*Rissoa subeostulata*, Schwarz Mediterranée

Each shell was placed in vertical position under the microscope by fixing the shell base with plasticine. Total numbers of whorls, ribbed whorls and terminal ribs per whorl, were counted according to Verduin's (1982) methods. Then each protoconch was photographed from above using an Olympus® CAMEDIA C-7070 WZ digital camera. Shells were then positioned, with the help of plasticine, with their vertical axes parallel to the observation plane and pictures were taken of the teleoconchs. Digitized images were opened in Adobe® Photoshop CS2 image editor software. Using measurement tools provided by the software (appropriately calibrated), total shell length (L), the diameter of the protoconch nucleus (d) and the diameter of the first half whorl (D_0) were measured, according to Verduin's (1985) methods. Furthermore, the apex type ($=D_0 + 0.72d$) was calculated aecording to Verduin (1985). Using the statistical software SPSS v.15 (© SPSS Inc., 2006), a discriminant function analysis was performed on the data matrix obtained, in order to detect significant differences between the type specimens of *R. panhormensis* and shells of *R. guerinii* (treating the four lots as a single group).

Live-collected Material: Living material was sampled on the rocky shore of Santa Tecla, Sicily (Mediterranean, Ionian Sea) at 1–5 m depth in Apr 2006. About 0.03 m^3 of the red alga *Pterocladiella capillacea* (Gmelin) Santelices and Hommersand was collected by SCUBA diving during each sampling. Collected material was immersed in seawater and transferred to the laboratory. The total amount of sampled material was partitioned into 20 subsamples that were washed for no more than 5 min in a tank containing 5 l of 50% seawater. The osmotic shock provided forced all vagile fauna to detach from the algal thalli and to fall on the bottom of the tank, from where specimens were easily collected and returned to seawater. After recovering from the osmotic shock, live

mollusks were sorted and identified under a Wild Makroskop M420 stereoscopic mieroscope. Specimens of *Rissoa guerinii* and five specimens referable to *R.* cf. *panhormensis* were picked up from the sorted material. Some of them were placed in running seawater at 18°C and provided with fresh *P. capillacea* talli; others were preserved in 80% ethanol. After eaeh sampling, beached detritus was also eollected on the beach facing the sampling site. This yielded 18 empty shells belonging to *R.* cf. *panhormensis*. The relative proportion of specimens of *R.* cf. *panhormensis* and speeimens of *R. guerinii* was about 3/100 in samplings of both living and dry material.

Head-Foot: Adult living speeimens of both *R.* cf. *panhormensis* and *R. guerinii* were plaeed in a Petri dish with seawater under a Leica Z16 APO stereoscopic mieroseope. Shells were held with forceps and the snails attempted to crawl extending their foot completely, enabling the head-foot to be observed in detail. Images of shells and head-foot were taken and digitized using a Leiea DFC 300 FX video eamera and Leica Application Suite version 2.4.0 software. Color drawings were also made to better represent the color pattern of the head-foot.

Geometric Morphometry: We randomly choose 15 adult shells from ethanol preserved specimens of *R. guerinii* and 15 adult shells of *R.* cf. *panhormensis* (three from ethanol-stored material and the remaining 12 from collected empty shells). Shells were observed using a Leica Z16 APO stereoseopic microscope, and eolor images were taken and digitized using a Leica DFC 300 FX video camera and Leica Application Suite version 2.4.0 software. The shells were always placed in the same position, with the coiling axis in vertical position and the aperture on the same plane as the objective (Carvájal-Rodríguez et al., 2005). Using the software tpsDIG2 v. 2.10 (Rohlf, 2007a), 19 landmarks (LM) were established (Figure 1). LM1 is the apex of the shell; LM2, LM4 and LM6 are placed on the right border of the profile at the beginning of the three last complete whorls. LM15, LM17 and LM19 are the corresponding landmarks on the left border of the profile. LM3, LM5, LM16 and LM18 mark the intermediate position respectively between LM2 and LM4, LM4 and LM6, LM15 and LM17, LM17 and LM19 along the curvature of the whorl; LM8 is at the lower suture of the last complete whorl and LM7 marks the intermediate position between LM6 and LM8 along the eurvature of the whorl. LM9 is the most external position in the upper part of the outer lip; LM10 and LM12 are the most external positions respectively in the external right and left part of the outer lip; LM11 is the lowest point at the base; LM14 is the most external point in the last whorl at the left profile of the shell; LM13 is the profile point between LM12 and LM14 (closest to LM7). As deseribed in Carvájal-Rodríguez et al. (2005) the matrix of raw coordinates generated by tpsDIG2 was used in tpsRelw v.1.45 (Rohlf, 2007b) to compute shell size (CS), uniform (U1 and U2) and non-uniform (several relative warps, RWs) shape components for each specimen. Classical parametric tests were performed on the obtained variables by the SPSS/PC package v. 15.0.

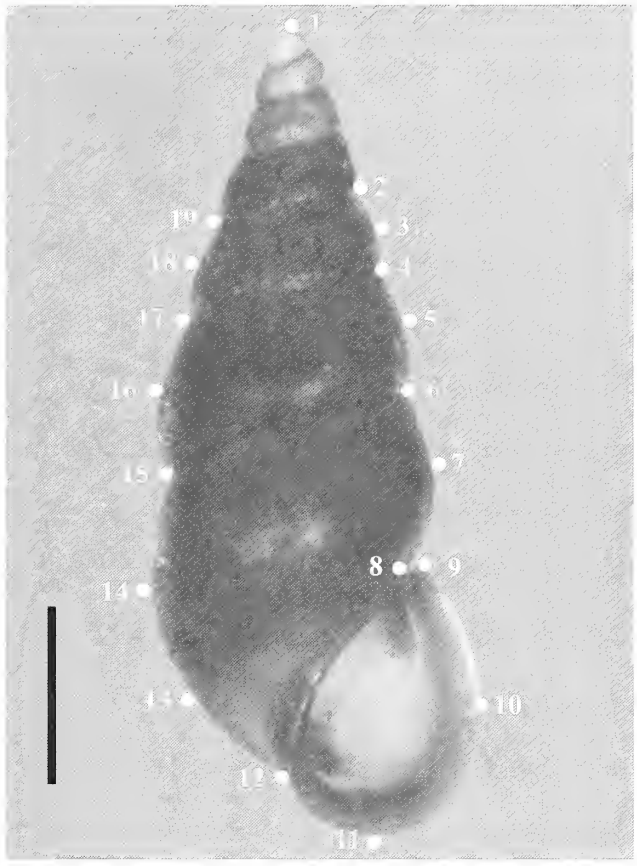


Figure 1. A shell of a living *Rissoa* cf. *panhormensis* from S. Tecla showing the placement of the 19 landmarks used for geometric morphometric analysis. Scale bar = 1 mm.

Molecular Systematics: Thirty-five live *R. guerinii* and five live *R.* cf. *panhormensis*, from the Santa Teela samples, were used for DNA analysis. The color pattern of each specimen was recorded before processing. The shell of each specimen was broken in a mortar and the entire organism was homogenized in a 1.5 ml eppendorf tube using 150 µl 2x CTAB extraction buffer (50 mM Tris HCl [pH 8.0], 0.7 M NaCl, 10 mM EDTA, 1% CTAB, 0.4% β-mercaptoethanol) with the addition of 10 µl of Proteinase K. DNA was extracted using standard CTAB protocol (Doyle and Doyle 1987) with one extra wash in phenol:ehloroform:isoamylalcohol (25:24:1) and one in chloroform:isoamylalcohol (24:1) in order to eliminate polysaccharides. Two mitochondrial DNA markers were amplified by PCR: (1) a 337 bp fragment of 16S rRNA was amplified in a 20 µl final volume containing 1 µl template DNA, 2 µl of 10X Roche diagnostic PCR reaction buffer, 2 µl dNTPs 10X (2 mM), 0.8 µl of eaeh primer (20 pmol/µl), 1 µl Biogem Taq polymerase (3 u/µl), 0.2 µl BSA. The primers were (designed with Oligo v. 6.71 software), U37 (5'-AGAGAATTACGCTGTTATCC

CTGT-3') and L373 (5'-AGAGAATTACGCTGTTATCC CTGT-3') with a target length of 360 bp; PCR conditions were: 94°C for 5 min, 40 cycles of 94°C for 1 min, 50.1°C for 1 min and 72°C for 1 min and a final elongation step of 7 min at 72°C; (2) a 372 bp fragment of COI was amplified in a in 20 µl final volume containing 20 to 50 ng template DNA, 2 µl of 10X Roche diagnostic PCR reaction buffer, 2 µl dNTPs 10X (2 mM), 1 µl of each primer (20 pmol/ µl), 1 µl Biogem Taq polymerase (2.5 u/µl), 0.2 µl BSA; the primers were, 59R-COI (forward: 5'-ATTGGTGGCTTTGGAAATTG-3') and 59L-COI (reverse: 5'-GATAGGGTCACCACCTCCTG-3'; Panico and Patti, 2005) with a target length of 450 bp; PCR conditions were: 94°C for 5 min, 40 cycles of 94°C for 1 min, 45°C for 30 sec and 72°C for 45 sec and a final elongation step of 7 min at 72°C.

PCR products were separated by gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions. Purified products were sequenced on a Beckman Ceq 2000 automatic sequencer, using a Dye-terminator cycle sequencing kit (Beckman) according to manufacturer's instructions. Sequences were assembled using the DNASTAR computer package (Lasergene), supplied with the Beckman sequencer. Sequences obtained for different marker from the same individual were concatenated in Bioedit v. 5.0.6 (Hall, 1999), treated as single sequence and aligned with CodonCode Aligner v. 1.6.3 (CodonCode Corporation, Dedham, MA), using ClustalW (Thompson et al., 1994) alignment method. The alignment was refined by eye. For all samples, both forward and reverse strands were analysed. Genbank accession numbers range from GU177879 to GU177963 for the 16S gene and from GU177964 to GU178011 for the COI gene.

The concatenated sequences were subjected to Maximum Parsimony and Maximum Likelihood tree reconstruction using PAUP* v. 4.04 (Swofford, 2003). *Rissoa labiosa* (Montagu, 1803) was used as the outgroup (Genbank accession numbers: AY676128 for COI and AY676117 for mt16SrRNA). The program Modeltest version 3.06 (Posada and Crandall, 1998) was employed to selected HKY + I model for ML analysis. Trees were computed with 1000 bootstrap replicates. Bremer support values (Bremer, 1994) were used in conjunction with bootstrap. A reduced median joining network (MJ) (Bandelt et al., 1999) was obtained with the software Network v. 4.5 (Fluxus Technology).

RESULTS

Dautzenberg Collection Material: **Visual Observation:** The type specimens of *Rissoa panhormensis* were kept in a glass tube in a small cardboard box. The holotype was isolated from the paratypes and enclosed in a small plastic case. The bad state of preservation of the periostracum and the consistent presence of mineral concretions, visible on the surface and in the inside of some shells, indicated some degree of shell degradation. The types appeared to be very similar to specimens identified as *R. guerinii* from the lots Rca, Rcb, Rsm and Rcm (see Table 1). Paratypes were rather slender with a reduced number of ribs often showing a high degree of bluntness towards the earlier whorls. This feature of the ribs was exacerbated on the spire of the holotype, giving this specimen the most peculiar aspect of the shells of type series. The shell pigmentation was typical of *R. guerinii:* white with brown spaces between ribs and a gray-violet apex. The tube with the holotype contained a label with two different handwritings: Verduin's original note: "*Rissoa panhormensis* Verduin/ Det.: Verduin, 1983" and an additional indication added in handwriting: "7 paratypes". Unfortunately, the staff of the malacological section of RBINS was unable to identify this handwriting. The box contained a larger label with the text: "*Rissoa panhormensis* VERDU1N/Palerme/Lemoro, Monts./PARATYPES". We were not able to find the original label with the inscription "*Rissoa guerinii*, Recl./ Palerme, Lemoro Monts." mentioned by Verduin (1985). This has probably been lost.

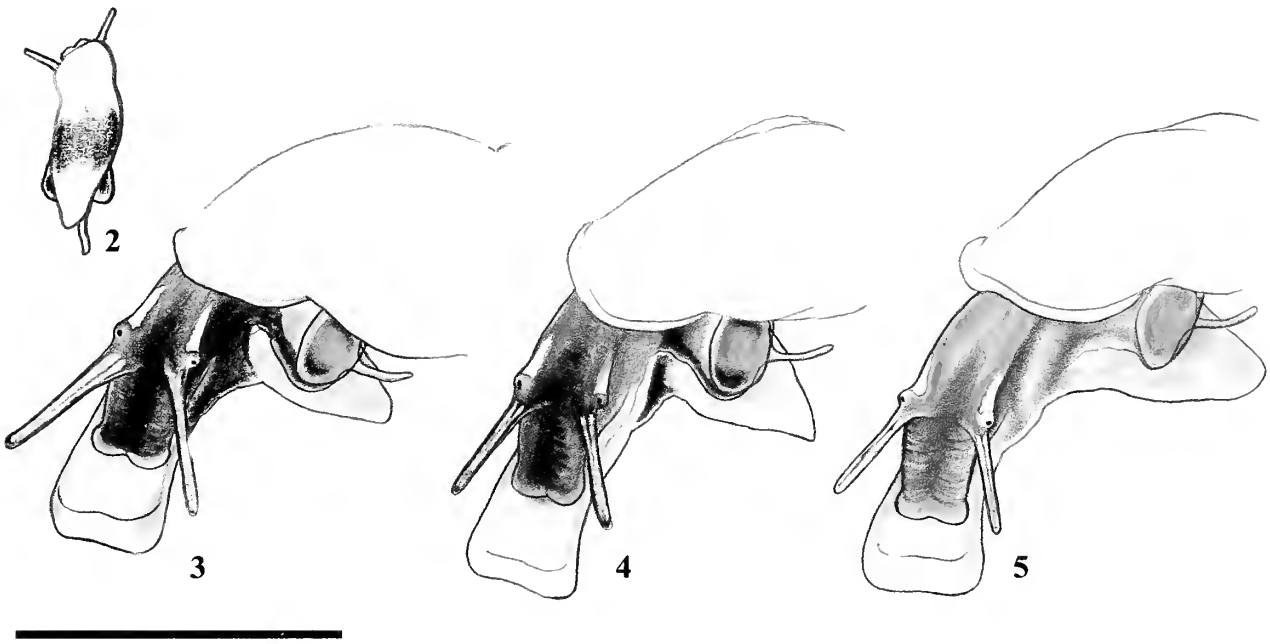
Morphometry: The eigenvalue of the discriminant function between the two species was 0.683, the canonical correlation 0.673 and Wilks' lambda (0.594) was not significant ($p > 0.05$), indicating a lack of any statistically valid separation between the two groups. As shown by Table 2, of the variables employed, the number of ribbed whorls was the most important one in distinguishing the two groups. Moreover, it was the only one revealing a consistent degree of correlation with the discriminant function (0.772).

Using the values of the discriminant function of each individual to predict its *a posteriori* species membership, 21 (84%) individuals, out of the total 25 used in the analysis, were attributed to the correct species and only four (16%) were erroneously *a posteriori* classified. Looking at species statistics, all shells of *R. panhormensis* were correctly assigned to this species. Only four (20%) specimens of *R. guerinii* were erroneously assigned to *R. panhormensis*, whereas 16 (80%) were assigned to the correct taxon.

Live-collected Material: **Head-foot:** The intensity of the pigmentation of the shell and head-foot enabled two color types of *Rissoa guerinii* to be distinguished, viz. typical *R. guerinii* and *R. guerinii* "var. *conspersa*" (Dautzenberg and Durouchoux, 1914; Figure 4 and 5). In both

Table 2. Shell variables in *Rissoa* spp. and their coefficients and correlation with the discriminant function.

	DF Coefficients	Correlation with DF
Ribbed whorls	1.020	0.772
Terminal ribs per whorl	–0.052	–0.278
D_0	–0.075	–0.236
d	–0.292	0.151
Length	–0.404	0.031



Figures 2–5. Pigmentation of the soft body parts of living specimens. **2.** Dark smudge of the middle part of the sole. **3.** *Rissoa* cf. *panhormensis*. **4.** *R. guerinii* "var. *conspersa*." **5.** *R. guerinii* (typical pigmentation). Scale bar = 1 mm. Drawings by Danilo Scuderi.

types, the foot was whitish and the middle part of the sole was stained brown (Figure 2), this latter feature being lighter in typical *R. guerinii* than in "var. *conspersa*." The snout was light brown in *R. guerinii* and darker brown in "var. *conspersa*." The margin of the distal portion of the snout and the rest of the head was yellowish in *R. guerinii* and light brown in "var. *conspersa*." The cephalic tentacles were whitish, but sometimes dark brown in "var. *conspersa*." A whitish spot behind the base of cephalic tentacles was always present. The body pigmentation of *R.* cf. *panhormensis* was similar to that of *R. guerinii* "var. *conspersa*," with a slight tendency to be darker (Figure 3). Table 3 summarizes the comparison among the different pigmentation patterns.

Geometric Morphometry: Table 4 shows the percentages and a descriptive statistical summary of the relative score for CS, the two uniform components and the first 8 RWs, explaining more than the 91% of the overall variation. Table 5 shows the results of the allometric analysis for shell shape measurements conducted by stepwise multiple regression analysis for centroid size (as dependent variable) and two uniform and 29 non-uniform measurements, as independent variables. The F-test of the regression analysis was significant ($p < 0.05$) and only one relative warp, RW3, contributed significantly to the regression model on the centroid size (Beta = –0.406). Centroid size, uniform components and only the first eight relative warps were considered in the analysis of variance (ANOVA) performed to evaluate the significance of differences in size and shape variables. Shells of *R. guerinii* and *R.* cf. *panhormensis* differed significantly in U1 ($p < 0.001$), RW2 ($p < 0.001$ and RW6 ($p < 0.05$). The cumulative results of the analysis are shown in Table 4. The significance level obtained for the corrected analysis (ANCOVA) with centroid size as covariate was not maintained for the difference in RW6, but was only slightly affected for RW2 and for U1 (both with $p < 0.05$). In addition, a significant difference was found between the two groups also for RW3 ($p < 0.05$). Table 4 shows these results.

Table 3. Summary of the observation on the pigmentation features in the specimens of *Rissoa* spp. considered.

Feature	*R. guerinii*	*R. g.* "var. *conspersa*"	*R.* cf. *panhormensis*
Foot colour	whitish	whitish	whitish
Sole stain colour	light brown	dark brown	dark brown
Snout colour	light brown	dark brown	dark brown
	yellowish distally	light brown distally	light brown distally
Head colour	yellowish	light brown	light brown
Cephalic tentacles colour	whitish	whitish or dark brown	whitish or dark brown
Tentacular spot colour	whitish	whitish	whitish

Table 4. Descriptive statistical summary and results of ANOVA and ANCOVA for the main shell size and shape variables between *Rissoa guerinii* and *R.* cf. *panhormensis*. $^{**}p < 0.05$, $^{***}p < 0.001$, ns = non significant.

Measure		CS	U1	U2	RW1	RW2	RW3	RW4	RW5	RW6	RW7	RW8
Variance explained					39.3%	27.5%	7.3%	5.1%	4.1%	3.2%	2.5%	2.0%
R. guerinii	Mean	1021	–0.010	–0.002	0.002	–0.017	–0.005	0.000	–0.002	0.004	0.001	–0.001
	St. dev.	122	0.014	0.010	0.039	0.025	0.017	0.014	0.010	0.011	0.011	0.009
R. cf. *panhormensis*	Mean	996	0.010	0.002	–0.002	0.017	0.005	0.000	0.002	–0.004	–0.001	0.001
	St. dev.	89	0.013	0.007	0.038	0.029	0.014	0.014	0.014	0.008	0.008	0.008
ANOVA		0.41^{ns}	18.26^{***}	1.47^{ns}	0.06^{ns}	11.80^{***}	2.49^{ns}	0.04^{ns}	0.64^{ns}	5.52^{**}	0.38^{ns}	0.34^{ns}
ANCOVA			9.09^{**}	3.14^{ns}	0.56^{ns}	5.69^{**}	3.84^{**}	0.50^{ns}	0.89^{ns}	3.31^{ns}	0.30^{ns}	1.65^{ns}

The eigenvalue of the stepwise discriminant function between the two species, calculated for all 29 RWs, was 4.033, the canonical correlation 0.895 and Wilks' lambda (0.199) was highly significant ($p < 0.001$), indicating a good separation between groups. Seven shape variables contributed to the discriminant function (RW2, RW6, RW13, RW3, RW27, RW16, and RW25). The standardized coefficient matrix (Table 6) shows the relative importance of the independent variables in determining the standardized canonical discriminant function. The mean values of the discriminant function for the two groups were –1.940 for *R. guerinii* and 1.940 for *R.* cf. *panhormensis*. Using the individual values of the discriminant functions to predict *a posteriori* species memberships, 26 (86.7%) individuals out of 30, were assigned to the correct species, leaving only 4 (13.3%) that were erroneously classified. Looking at species statistics, 13 (86.7%) specimens of *R. guerinii* were correctly assigned to this species and only two (13.3%) were assigned to *R.* cf. *panhormensis*. The same percentages of correctly/erroneously classified specimens of *R.* cf. *panhormensis* were observed. In Figure 3 the thin plate spline representation allowed us to interpret in geometric terms the positive (characteristic of *R.* cf. *panhormensis*) and negative deviations (characteristic of *R. guerinii*) values for the most significant non uniform shape variable, RW2, between the two species.

Molecular Phylogeny: After combining the COI and 16S rRNA sequences, a concatenated sequence of 709 bp was obtained, yielding 20 different haplotypes 18 of which involved exclusively *R. guerinii*, while the two remaining ones were shared by both *R. guerinii* and *R.* cf. *panhormensis*. The topologies of the MP and ML trees (Figure 4) were comparable: four haplotypes of *R. guerinii* occupy nested basal positions in the tree, while the remaining 16 haplotypes, belonging to *R. guerinii* and *R.* cf. *panhormensis*, form a terminal clade supported by bootstrap values of 70–75 (MP and ML respectively). The MJ network (Figure 5) confirmed the presence of a common haplotype occurring in 19 specimens (n = 19) of both *R. guerinii* (n = 15) and *R.* cf. *panhormensis* (n = 4), one haplotype occurring in two specimens of *R. guerinii*, 17 unique haplotypes, and one haplotype that occurred in one specimen of each species. 14 haplotypes are separated by 1–5 differences from the main one, but four showed larger distances (27, 25, 23 and 15 respectively).

Table 5. Multiple regression model to test allometry for the non-uniform shell shape variables in *Rissoa* spp.

Multiple regression		Variables in the model	
r^2	F	Name	Beta
0.165	5.6^{**}	RW3	-0.406^{**}

$^{**}P < 0.05$, $^{***}P < 0.001$.

DISCUSSION

Dautzenberg Collection Material: **Visual Observation:** Monterosato (1884) considered *R. costulata* Alder, 1844, and *R. subcostulata* Schwarz, 1864, as synonyms of *R. guerinii*, so we checked if the original label for *R. panhormensis* might be found accompanying a lot of those two taxa. Only two labels were found to have these characteristics: that of the already mentioned sample Rcm (*Rissoa costulata/Medit./Monts.*) and one found in the bottom of a box containing lots of *R. guerinii* coming mainly from French coasts, whose inscription was: "*Rissoa costulata, Alder*/Palerme/Lemoro Monts.". While it is possible that one of these could be the original label accompanying the type lot of *R. panhormensis*, it is unlikely as it does not correspond exactly to the wording given by Verduin. We are unsure if the type material originally constituted a single separated sample or whether it was a part of a larger sample from which Verduin isolated seven specimens. However, there was a strong resemblance between *R. panhormensis* type material and the four lots referable to *R. guerinii* (Rca, Rcb, Rcm and Rsm) suggesting that these lots may at least share a common geographic origin.

Table 6. Standardized coefficient matrix showing the relative importance of the shape variables in *Rissoa* spp.

	Discriminant function
RW2	1.145
RW6	–0.929
RW13	–0.702
RW3	0.686
RW27	0.683
RW16	0.581
RW25	0.460

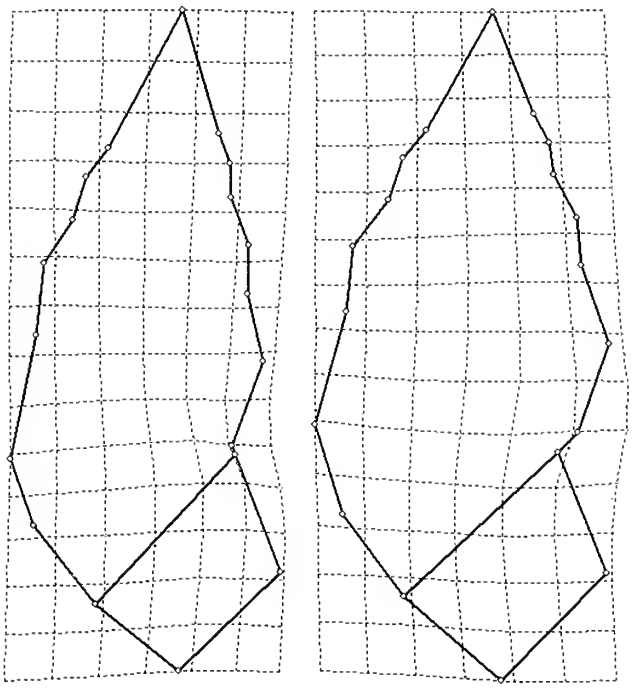


Figure 6. Thin plate spline representations for RW2, showing the deformation of the grid for the average values of *Rissoa guerinii* (left) and *R.* cf. *panhormensis* (right).

Another important observation was the unrepresentative nature of the holotype with respect to the paratype series. This shell, rather than summarizing the average characteristics of the type series, represents instead the most extreme variant with a nearly total lack of ribs. The illustration provided by Verduin for this shell is not of good quality and this has contributed to perpetuating the idea that *R. panhormensis* is a ribless species. This characteristic is reflected in the shells of our sampled specimens, here referred to as *R.* cf. *panhormensis*.

Morphometry: Verduin (1985) stated that the samples containing the type specimens were probably dredged, because Monterosato used to obtain detritus from fishing nets and then pick out and classify the interesting shells. Using this method he selected the samples that he retained for his collection. In view of this possible lack of randomness in the samples obtained from Monterosato, we could not use them to reliably infer interpopulational or interspecific differences between samples. Our morphometric investigation arose from the observation that the type material of *Rissoa panhormensis* strongly resembles *R. guerinii*, despite the claim of a morphological (and morphometric) distinction between both taxa (Verduin, 1985). This claim was mainly based on alleged differences in the size of the apex and differences in the number of shell ribs. However, our discriminant analysis of three protoconch variables (d, D_0, and At) did not support significant differences between the size of the apex of *R. panhormensis* and *R. guerinii*, and both belong to the larger apex category (At > 0.235 mm). Moreover, we

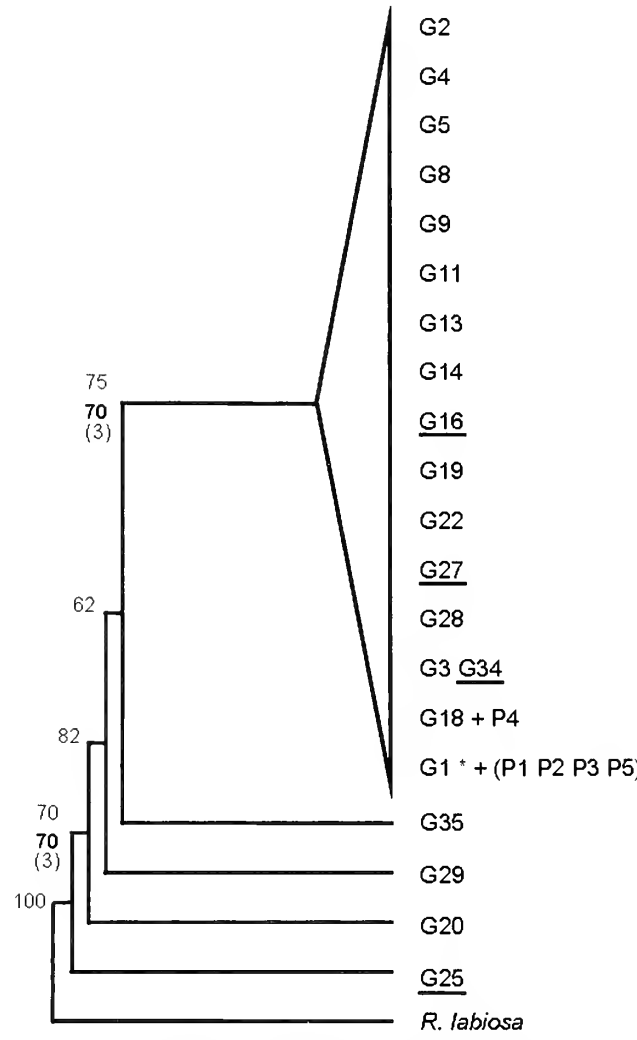


Figure 7. Maximum Likelihood tree obtained from concatenated sequences of 16S and COI genes obtained for *Rissoa guerinii* and *R.* cf. *panhormensis*. A number of 1000 bootstrap replicates were performed and its values (if above 50%) are shown on the nodes. Bootstrap values for Parsimony are in bold; in brackets the Bremer index values. Letter G refers to *R. guerinii* haplotypes, letter P to *R.* cf. *panhormensis* haplotypes. G1* = G1, G6, G7, G10, G12, G15, G17, G21, G23, G24, G26, G30, G31, G32, G33. Specimens of *R. guerinii* "var. *conspersa*" are underlined.

observed apex types exceeding Verduin's arbitrary, species specific "cut-off values" within *R. guerinii* (Criscione and Patti, submitted.). Hence, if only the dimensions of the apex were used to distinguish *R. panhormensis* (type material) from Dautzenberg's *R. guerinii* samples, then this would not result in a clear separation of both taxa. In other words: the *R. panhormensis* types were not the only specimens among Dautzenberg's *R. guerinii* material to have the larger type of apex.

Although number of whorls (N) and shell length (L) show considerable intraspecific variation, they nevertheless often reveal consistent interspecific differences (F.C., pers. observ.). However, our statistical analysis revealed

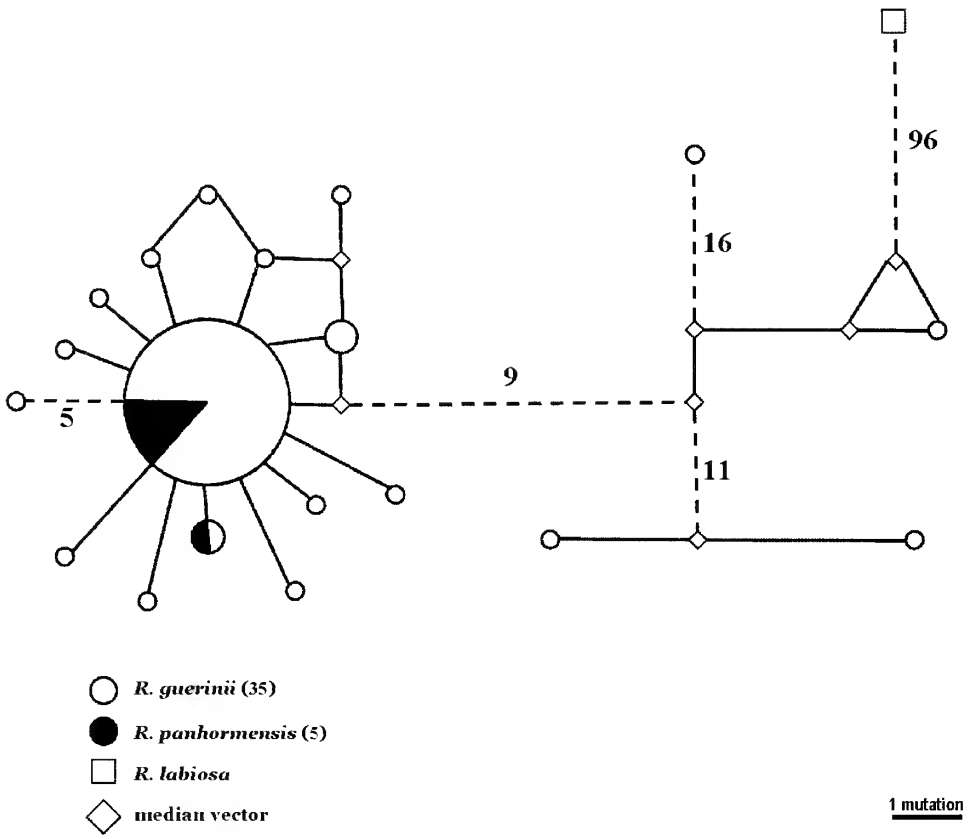


Figure 8. Median Joining Network drawn from concatenated sequences of 16S and COI genes obtained for *Rissoa guerinii* and *R.* cf. *panhormensis*. In round brackets the number of sequences employed. Dashed lines represent higher number of mutations (values reported nearby).

that the differences between the samples for these two characters were not significant. Finally, although Verduin (1985) emphasized the low number of radial ribs of *R. panhormensis*, it is unclear to us as to whether this related to a smaller number of ribbed whorls or a smaller number of ribs on the last whorl. In this study only the total number of ribbed whorls (RW) can be used to discriminate the two groups and this may be what Verduin really meant as it appears to be the only difference by which *R. panhormensis* and *R. guerinii* can be separated.

Living Material: **Geometric Morphometry:** The ANOVA did not reveal any significant difference in size (CS) between the two groups, confirming our non-casual observations of the Dautzenberg collection samples. A high level of significance was instead observed for the first of the uniform shape components, U1, which, accounting for compression-dilation deformations, may be interpreted as indicating that *Rissoa* cf. *panhormensis* has a more slender shell compared to *R. guerinii*. Analysis of variance showed a highly significant difference ($p < 0.001$) between the two groups in the second non uniform shape variables (RW2) and a significant difference ($p < 0.05$) for the sixth relative warp (RW6). The significance for U1 remained unaltered when correcting the analysis for CS (ANCOVA), while that of RW2 decreased to significant ($p < 0.05$) and that of RW6 was not significant, indicating that the shape difference explained by those variables was dependent on size. The difference in RW3, was not significant in the ANOVA, but became significant ($p < 0.05$) in the ANCOVA. Despite these variations, RW2 always showed the lower p value, which means that the two groups mostly differ on this shape variable independently from the correlation between shape and size.

The discriminant function calculated from all the non uniform shape variables, was successful in morphometrically discriminating the two groups. RW2 was the most important variable in determining the distinction between *Rissoa guerinii* and *R.* cf. *panhormensis*. The mean values of this variable for each of the two groups (positive for *R.* cf. *panhormensis* and negative for *R. guerinii*) were plotted in a tps representation (Figure 3). The plot showed that that variable RW2 is a reflection of the most obvious discernable shell shape difference. This comprised the slenderer aspect of *R.* cf. *panhormensis*, contributed by a consistently narrower penultimate whorl (represented by the contraction of the corresponding zone of the grid) than that of *R. guerinii*. A similar interpretation of a single relative warp

resulting in shell slenderness has also been reported by Carvájal-Rodríguez et al. (2006) in *Nassarius*. In this case, however, the absence or reduction of axial ribs may have affected the representation of horizontal dimensions, resulting in this visual difference. The flatter aspect of the whorls of *R.* cf. *panhormensis* is also linked to the lack of ribs. Also noticeable is the opposite relative displacement of landmarks 8 and 9 (Figure 6) and the subsequent modifications of the grid. In *R.* cf. *panhormensis*, LM 9 is overlapped to a greater extent by LM 8 than in *R. guerinii*. This is because of the presence of ribs on the penultimate whorl of *R. guerinii*, which partly overhang the posterior part of the outer lip, giving the impression of a less protruding peristome. In contrast, the lack of ribs in *R.* cf. *panhormensis* accounts for the more protruding peristome of this morph.

Head-foot: In the genus *Rissoa*, the pigmentation of head-foot is often an important species-specific character (Fretter and Graham, 1978). In addition, *Rissoa* species also often differ in the relative proportions of head-foot components (cephalic tentacles, snout and anterior part of the foot) (D.S. pers. observ.). One cannot use these characters to distinguish *R.* cf. *panhormensis* from *R. guerinii* "var. *conspersa*."

Molecular Systematics: The combined 16S and COI sequence data showed that *R. guerinii* and *R.* cf. *panhormensis* cannot be separated.

Radial Ornamentation and Species Distinction: Our results indicate that reduced radial ornamentation is the only distinguishing feature between *R. panhormensis* and *R. guerinii*. Problems with using the number of ribs for species distinction in the genus *Rissoa* are not new. There is evidence to show that rib number can be influenced by environmental conditions. For example, in fluctuating salinity, *R. parva* (Da Costa, 1778) can show reduction in radial ornamentation (Wigham, 1975; Verduin, 1976; Warén, 1996). Moreover, several unpublished field observations made by the authors on other species of *Rissoa*, living in low salinity environments (sheltered bays, coastal seagrass meadows), revealed the tendency in entire populations (*R. similis*) or a small number of individuals in a population (*R. auriscalpium*, *R. labiosa*) to lack axial ribs. It appears that this latter situation is the case with *R. guerinii*. There is an abundant inflow of freshwater in our sampling site as well as other parts of the Sicilian Ionian coast. Where those conditions occur, it seems possible that *R. guerinii* might develop "smooth" morphotypes, as in our sample locality.

This view is in agreement with the idea of *R. guerinii* as a highly polymorphic and/or plastic taxon. Indeed, besides *R. panhormensis*, there are other Mediterranean species of *Rissoa* (e.g. *R. decorata* Philippi, 1846, *R. torquilla*, Pallary, 1912, and *R. frauenfeldiana* Brusina, 1868) whose shells are quite similar to *R. guerinii*. It would not be surprising if further studies show that those species are also ecophenotypes of *R. guerinii*.

CONCLUSIONS

We find that *Rissoa* cf. *panhormensis* is a rare morphotype of *R. guerinii* characterized by reduced development of radial ornamentation, and that, as such, it should be considered to be a junior synonym of this latter species.

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Three new species of *Humboldtiana* (Gastropoda: Pulmonata: Humboldtianidae) from Mexico

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ABSTRACT

Three new species of *Humboldtiana* from the states of Hidalgo, Nuevo León and Sonora, Mexico are described. By the combination of internal and external morphological features two belong to the *Humboldtiana buffoniana* species group and one belongs to the *Gymnopallax* subgenus.

Additional keywords: Taxonomy, land snail, pulmonate, neotropical region

INTRODUCTION

Humboldtiana comprises approximately 50 species distributed from South Texas to Central Mexico. Low vagility and dispersal abilities are reflected in high levels of endemism and highly restricted distributions (Thompson and Brewer, 2000). The genus is characterized by the presence of four dart sacs surrounding the vagina (each one bearing two dart bulbs); four dart glands form a ring around the vagina, the spermathecal duct with a caecum in the distal end, the penis containing a verge and flagellum moderately long. Variations to this general pattern have lead to the proposal of six subgenera: *Polyomphala, Humboldtiana, Oreades, Gymnopallax, Clydonacme*, and *Aglotrochus* (Thompson and Brewer, 2000; Thompson, 2006), and three species groups within subgenus *Humboldtiana: H. buffoniana group, H. texana* group *and H. bicincta* group (Burch and Thompson, 1957; Thompson and Brewer, 2000). Repository institutions for type material are: CNMO, Colección Nacional de Moluscos, Instituto de Biología, UNAM, Mexico; DP, Colección Malacológica de la Subdirección de Laboratorios y Apoyo Académico del INAH, Mexico; UF, Florida Museum of Natural History, Gainesville, Florida. Description of the new species is based on the holotype and two paratypes; in all cases first measurements are from the holotype and measurements in parentheses are from paratypes 1 and 2 respectively.

SYSTEMATICS

Family Humboldtianidae Pilsbry, 1939

Genus *Humboldtiana* von Ihering, 1892

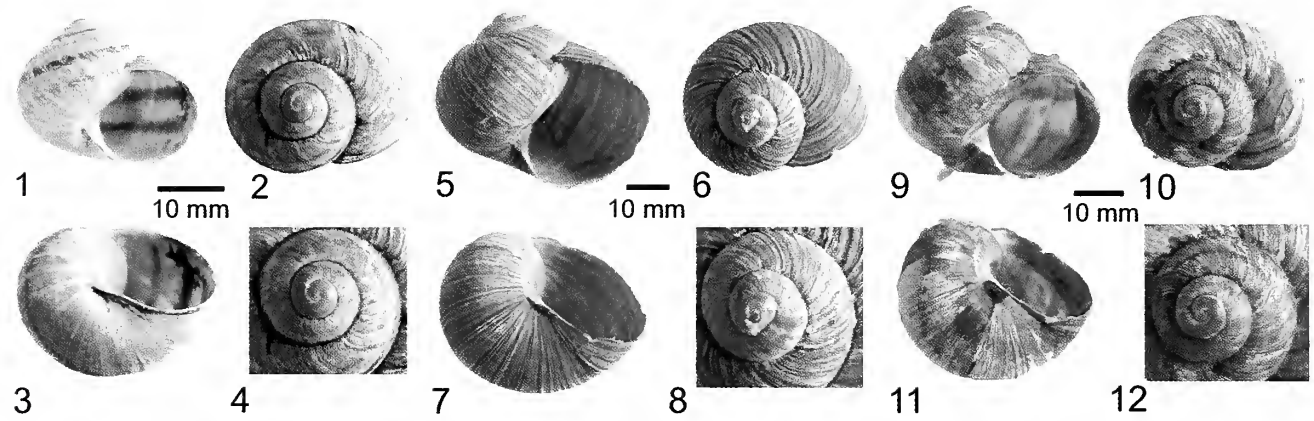
Humboldtiana salvialtispanica new species
(Figures 1–4, 13)

Diagnosis: A small *Humboldtiana*, with a pale brown shell bearing three chestnut to dark brown bands clearly visible on the internal surface of the shell. The subglobose shell and the glands just above dart sacs separate the new species from *H. edesma*, the only other species known to also have an almost indistinguishable atrium.

Description: Shell (Figures 1–4): Shell globose, external lip slightly thickened, pale brown, with three chestnut to dark brown bands, second band wider than others, although in one specimen third is widest, 4.2 whorls (4.1, 4.1). Embryonic shell caramel in color, with 1.5 whorls (1.6, 1.5), first whorl without sculpture then with almost imperceptible growth lines, followed by well-marked growth lines. Sculpture of rest of shell constituted of white to pale-brown well-marked growth lines with almost uniformly distributed ovate granules. Umbilicus almost covered by aperture margin, granules faint in that area. Very thin, transparent callus. Shell height: 22 mm (25, 25); shell diameter: 26 mm (30, 29); aperture height: 17 mm (20, 18); aperture diameter: 17 mm (21, 17).

Reproductive Anatomy (Figure 13): Penis short and stocky, almost roundish below mid line, 7.8 mm (13.65, 11.8), interior of penis with two longitudinal folds, with large and broadened verge that cover entire penis cavity, verge composed of two large triangular lobes attached to penis wall by two rounded, smaller (half as big as larger lobes) lobes located at end of penis. Penis retractor

[†] *In absentia.*



Figures 1–12. Figures 1–4. *Humboldtiana salviahispanica* new species, holotype, DP 691. **Figures 5–8.** *Humboldtiana thompsoni* new species, holotype, DP 692. **Figures 9–12.** *Humboldtiana ootamorum* new species, holotype, CNMO 1188.

muscle 6.8 mm (6.45, 16). Epiphallus long, cylindrical, measuring 16 mm (21, 11.5). Atrium very short, almost indistinguishable, measuring 1.3 mm (1.5, 1.5). Vagina cylindrical, expanded to darts region, measuring 7 mm (10, 6), four dart sacs of approximately same size: ds_1, 1.2 mm; ds_2, 1 mm; ds_3, 1.2 mm; ds_4, 1.5 mm (1.7, 1.5, 1.5, 1.5) (1.8, 1.6, 1.8, 1.9). Glands form a complete ring just above dart sacs, maximum height 2 mm (2.6, 3). Spermathecal duct measuring 40 mm (64, 85); spermathecal caecum measuring 5 mm (7.2, 7.9); spermatheca adhering to albumen gland, enlarged, sac-shaped, measuring 7 mm (5.5, 6.3). Flagellum relatively short at 35 mm (42, 47), about 1.68 (1.48, 2.41) times the combined length of the penis + epiphallus.

Type Material: HOLOTYPE: DP 691; collected 29 June,1995, by Ana B. Mancera and Gabriel Villegas Guzmán. PARATYPES: UF 376799 (1), CNMO 2731 (1); same data as the holotype. All from type locality.

Type Locality: HIDALGO: 4.1 km south and 6.8 km west of Huichapan, Hidalgo, 2290 m alt. (20°20'15"N, 99°42'45" W)

Remarks: This species belongs to the *H. buffoniana species* group, where glands are just above dart sacs. Short atrium is also present in *H. edesma*, but, in *H. edesma* the glands are clearly separated from dart sacs. On sight, shell resembles *H. pinicola*, however, Huichapan specimens are smaller, with fewer whorls and with lighter bands, furthermore, *H. pinicola* presents an atrium as long as penis, the epiphallis short and stout, whereas spermathecal duct and spermatheca are very short.

Etymology: The type locality is known to the Nahoas people as "Hueychapan," which means "On the chía water or on the chía river"; the epithet *salviahispanica* derives from *Salvia hispanica*, the scientific name of the chía plant, and is here used as a name in apposition.

Humboldtiana thompsoni new species
(Figures 5–8, 14)

Diagnosis: A large shell with almost imperceptible bands, interior of the shell slightly iridescent. The pear-shaped penis and the very long flagellum distinguish it from other members of the *Humboldtiana buffoniana* "species group".

Description: SHELL (Figures 5–8): Shell globose, external lip not reflected, light brown in color, bands lacking in holotype, second and third bands perceptible up

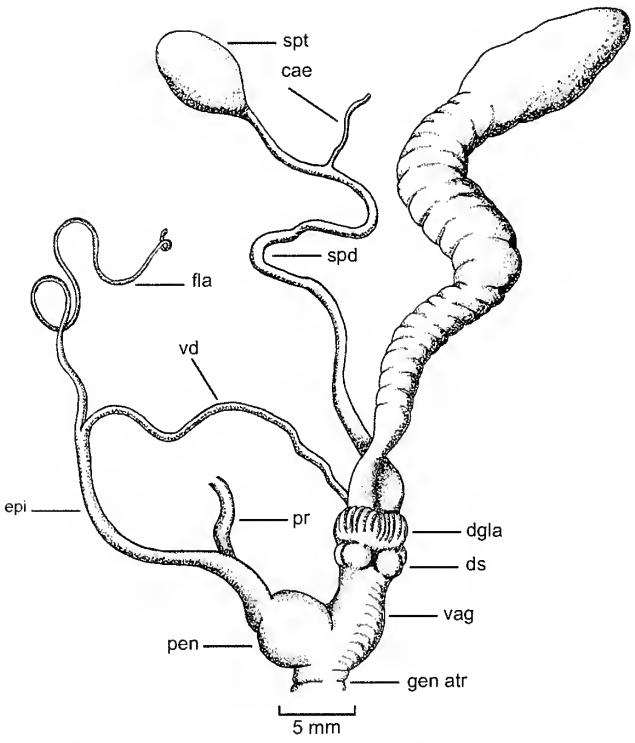


Figure 13. Reproductive anatomy of *Humboldtiana salviahispanica* new species, paratype, CNMO 2731. Abbreviations: **cae,** spermatheeal caecum; **dgla,** dart glands; **ds,** dart sacs; **epi,** epiphallus; **fla,** flagellum; **gen atr,** genital atrium; **pen,** penis; **pr,** penis retractor; **spd,** spermatheeal duct; **spt,** spermatheca; **vag,** vagina; **vd,** vas deferens.

to the third and first tenth of the body whorl in paratype UF376800 and perceptible until the first quarter of the body whorl in the paratype CNMO2732. In holotype, internal shell surface brown with slightly iridescent white patch, in paratype UF376800 internal surface of shell white, slightly iridescent, and in paratype CNMO2732 internal surface of shell white brown. In all specimens there are 4.1 whorls, embryonic shell cream colored with 1.2 whorls (1.25, 1.2), embryonic sculpture consisting of very thin growth lines that increase toward body whorl, with oblong papillae near suture. Rest of shell sculpture consisting of well-marked growth lines, white on a pale-brown background. Granules large, randomly distributed, increasing in size on subsequent whorls. Umbilicus narrow, completely covered by margin in holotype and paratype CNMO2732, and incompletely covered by margin in paratype UF376800.

Thin white callus. Shell height: 42 mm (30, 35); shell diameter: 49 mm (35, 43); aperture height: 35 mm (25, 29); aperture diameter: 34 mm (22, 25).

Reproductive Anatomy (Figure 14): The following description is based on the holotype and two paratypes. Penis varies from asymmetric pear-shaped to almost globose, measuring 10 mm (6.3, 9.3), interior of the penis with large, cylindrical verge. (Short conical verge in paratype CNMO2732), inner penis with four longitudinal folds. Penis retractor muscle 37 mm. Epiphallus short and stout, measuring 5.2 mm (6, 8), vas deferens uniformly slender. Cylindrical atrium, slightly elongated, measuring 4.5mm (6, 4). Vagina short, cylindrical, measuring 3.3 mm (2.2, 2.3). Four dart sacs of same size, each with one dart, dart bulbs not exposed, measuring 6 mm (4.7, 2.9). Glands short, forming ring just above dart sacs, maximum height 2.7 mm (2.9, 1.7). Spermathecal duct very long, diverging from uterus just above glands, mesuring 190 mm (133, 100), with short caecum, measuring 30 mm (13, #). Spermatheca varies from elongated sac-shaped to globose, adhering to anterior end of uterus-prostate, at base of albumen gland, measuring 84 mm (55, 44). Flagellum very long, measuring 232 mm (182, 218), 15.26 (14.79, 12.6) × combined length of penis + epiphallus.

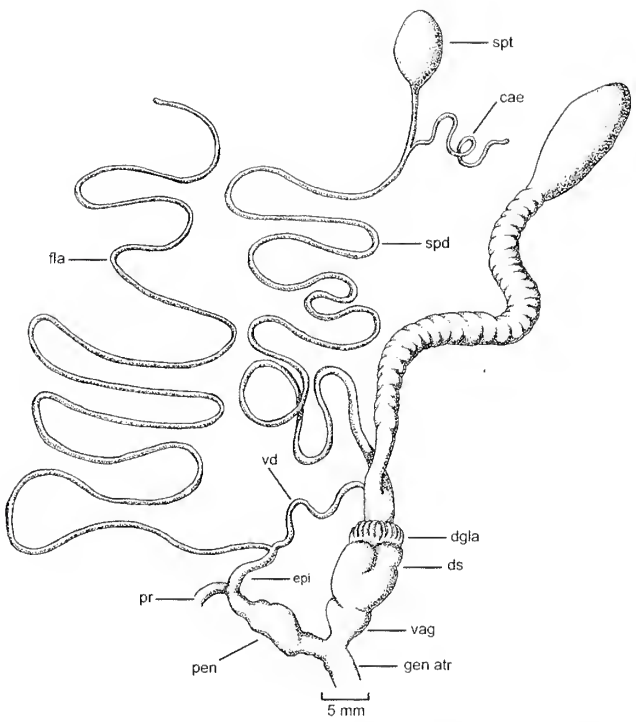


Figure 14. Reproductive anatomy of *Humboldtiana thompsoni* new species, holotype, DP 692. Abbreviations: **cae,** spermathecal caecum; **dgla,** dart glands; **ds,** dart sacs; **epi,** epiphallus; **fla,** flagellum; **gen atr,** genital atrium; **pen,** penis; **pr,** penis retractor; **spd,** spermathecal duct; **spt,** spermatheca; **vag,** vagina; **vd,** vas deferens.

Type Material: HOLOTYPE: DP 692; collected 15 April 1990, by Oscar J. Polaco. PARATYPES: UF 376800 (1), CNMO 2732 (1); all from type locality.

Type Locality: NUEVO LEÓN: Cañon de Carretas, 11.1 km north and 4.4 km west of San Josecito, 1740 m alt. (24°04'11" N, 99°56'58" W).

Remarks: The presence of four equal-sized dart sacs and the position of the dart glands indicates that this species belongs to the *Humboldtiana buffoniana* "species group." The extremely long flagellum was seen before only in the subgenus *Polyomphala*, although, members of this subgenus are characterized by exposed dart bulbs and depressed shells. The length of the flagellum in *H. thompsoni* is comparable only with that observed in *H. pilsbryi* (354 mm), but in this latter species, penis and epiphallus are cylindrical and the atrium is short and broadened (unpublished data).

Etymology: This species is dedicated to Dr. Fred G. Thompson, curator of the Mollusk Collection at the Florida Museum of Natural History, who kindly provided several samples for molecular studies of the genus.

Humboldtiana ootamorum new species
(Figures 9–12, 15)

Diagnosis: The brown-golden shell, the dart glands just above the darts sacs and the presence of granular sculpture distinguish the new species from other species of the subgenus *Gymnopallax*.

Description: Shell (Figures 9–12): Shell globose, external lip slightly thickened, brown-golden color, with white stains that impart an ash colored tint, with three continuous charcoal to dark-brown bands, slightly perceptible through the shell. Internal shell surface whitish, with 3.8 to 4.3 whorls. Embryonic shell pale brown to caramel color, 1.5 to 1.75 whorls, first whorl without sculpture, then with well-defined growth lines, with fine, uniform, granules that increase in size. Umbilicus completely covered by margin, where granules are less perceptible. Thin to moderate whitish callus. Shell

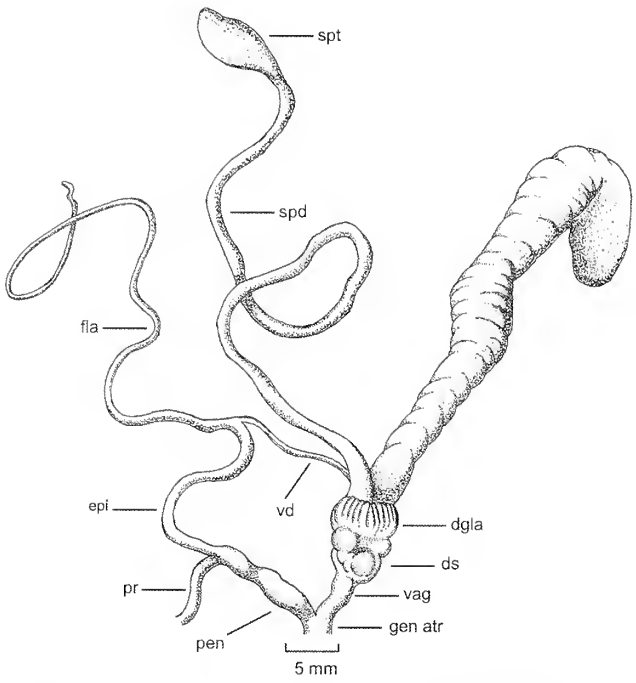


Figure 15. Reproductive anatomy of *Humboldtiana ootamorum* new species, paratype, DP 690. Abbreviations: **dgla,** dart glands; **ds,** dart sacs; **epi,** epiphallus; **fla,** flagellum; **gen atr,** genital atrium; **pen,** penis; **pr,** penis retractor; **spd,** spermathecal duct; **spt,** spermatheca; **vag,** vagina; **vd,** vas deferens.

height: 36 mm (29, 26); shell diameter: 38 mm (36, 27); aperture height: 28 mm (23, 20); aperture diameter: 29 mm (25, 17).

Reproductive Anatomy (Figure 15): Penis cylindrical, elongated, slightly expanding toward apex, although in one specimen short and uniformly broadened, measuring 9.75 mm (9, 8.25). Interior of penis with four longitudinal folds, with short and expanded verge that covers almost half of the length of the penis, verge formed by two superimposed tissue folds, one with three digitiform processes, middle process of upper fold longer than others, on lower fold all of same size. Behind verge, inner penis with thick crescent-shaped fold. Epiphallus cylindrical, slightly wider at base, measuring 24.6 mm (25.6, 22.5). Atrium short, measuring 2.4 mm (2.85, 2.25).Vagina tubular, elongated, measuring 5.7 mm (6.15, 4.9). Four dart sacs of approximately equal size, measuring 2.7 mm (3, 2.25), each with two exposed dart bulbs at base. Glands short, measuring 3.75 mm (2.4, 1.95), forming compacted ring just above dart sacs. Spermathecal duct elongated, measuring 87 mm (73, 67.5), spermatheeal caecum absent. Spermatheca sac-shaped, adhering to uterus-prostate, measuring 12.75 mm (7.65, 7.95). Flagellum relatively short, measuring 70 mm (52, 39), 2.03 (1.5, 1.26) × combined length of penis + epiphallus.

Type Material: HOLOTYPE: CNMO 1188; collected 15 August, 1998, by George M. Ferguson. PARATYPES: UF 376801 (1), DP 690 (1); all from type locality.

Type Locality: SONORA: Mesa el Campanero (=Mesa de Enmedio), Barranea El Salto (West side of Mesa), 2060 m alt., 28°21'20" N, 109°02'05" W.

Distribution: An immature specimen that resembles *H. ootamotum* was colleeted on "Arroyo La Pinosa, 9 km al Este del Puente del río Maicoba, Municipio de Yéeora, Sonora, Mexico, 1500 m alt., 28°24' 30" N, 108°43'30" W, CNMO 1189, colleeted 7 August, 2000, by George M. Ferguson", the shell is similar but the individual was not sexually mature, for the aforementioned we exclude this specimen of the description.

Remarks: The presence of two bulbs exposed at the base of the dart sacs, the absence of sculpture in the embryonic whorl, and the presence of a long spermathecal allocates this speeies to the subgenus *Gymnopallax*. It differs from the other species of that subgenus by its granular seulpture. Furthermore, in *Humboldtiana ootamorum* dart glands are just above dart saes, eompared with *Humboldtiana sylvania* and *Humboldtiana cicatricose*, where dart glands are widely separated from the dart sacs.

Etymology: The Pimas, early inhabitants of this region of Sonora, called themselves "o-otam," or "people of the river;" this species is named after this ethnieal group of Northwest Mexieo.

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We are grateful to José Luis Alvarado for take the photographs, to Fred G. Thompson for the eorrect derivation of the epithet *ootamorum* and to two anonymous reviewers for their useful comments. This work was partially funded by CONACYT projeet number 54719. This work is dedieated to the memory of Prof. Osear J. Polaco.

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Research Note

An aberrant sinistral *Conus* (Neogastropoda: Conidae) from the Miocene of Florida, USA

INTRODUCTION

Nearly all members of the hyperdiverse genus *Conus* typieally exhibit dextral, or right-handed, shell coiling. Sinistral, or left-handed, shell eoiling is a species-level charaeteristic of an extinct taxon—*Conus adversarius* Conrad, 1840—from the southeastern United States (see Hendricks 2009a, b), but sinistral coiling is otherwise known from fewer than 30 individuals from seven extant, typieally dextral species (Hendricks, 2009b). Given the tremendous interest and energy that has been put into the eollection of cone shells over the last several centuries, as well as the remarkable diversity of the genus (over 1,500 fossil and extant species; Röckel et al., 1995), these small numbers of confirmed reverse-coiled *Conus* are remarkable. Here we present the first reeord of an aberrant sinistral *Conus* fossil from an extinet species and briefly discuss its significanee.

MATERIALS, METHODS, RESULTS, AND DISCUSSION

The sinistral *Conus* fossil (Figure 1)—UF 137855, Florida Museum of Natural History, Division of Invertebrate Paleontology—was collected from the lower Miocene Chipola Formation (~18 Ma; Bryant et al., 1992; Jones et al., 1993) at Tenmile Creek, Calhoun County, Florida, USA (UF loeality CA020). Speeimen UF 137855 is broken and abraded, preventing us from making a definitive identification, but several features suggest that it is probably a specimen of the typieally dextral speeies *Conus vegrandis* Hoerle, 1976 (see Hoerle, 1976, table 1, for a listing of characters that separate *C. vegrandis* from co-oeeurring Chipola speeies). These features include: spire and body whorl outlines that are slightly sigmoid in profile; the presence of raised spiral cords on the anterior half of the body whorl; and the thin, ridge-forming carina on the shoulder of the body whorl noted by Hoerle (1976) in her original deseription of the species (this feature is not present in *C. adversarius* and negates the possibility that UF 137855 is an individual of that younger Plio-Pleistocene species). The apex of UF 137855 is eroded, preventing us from characterizing its protoconch and early postnuclear whorls. A paratype (UF 171658) of *C. vegrandis* from the same locality as UF 137855 is shown in Figure 2.

Hendricks (2009b) noted that all sinistral individuals of otherwise dextral extant taxa with known developmental modes belong to speeies with lecithotrophic larval development. He also showed that this was the case for *C. adversarius*, and was likely important to the initial origin and establishment of that species. One of us (JRH) observed the protoconchs (Figure 3) of five specimens of *C. vegrandis* using a FEI Quanta 200 seanning electron microscope at San Jose State University and, from the resulting images, measured the diameter of each protoconch, as well as its number of whorls (counted using the methodologies described in Jablonski and Lutz, 1980, and Tursch and Greifeneder, 2001, both of which gave similar results). On average, protoconchs of *C. vegrandis* had diameters of about 0.76 mm (range of about 0.73 to 0.82 mm) and about 1.9 whorls (range about 1.7 to 2.1 whorls). These data were then considered in the context of Shuto's (1974) model for predicting developmental mode based on protoconch diameter and number of whorls, which has been previously applied to *Conus* by Kohn and Perron (1994) and Hendricks (2009b). All five specimens fall within the lecithotrophic portion of Shuto's (1974) model (see Hendricks, 2009b, fig. 2), suggesting that *C. vegrandis* had that developmental mode. Thus, the association between sinistral shell eoiling and lecithotrophic development also appears to hold true for *C. vegrandis*.

Grande and Patel (2009) recently showed that the genes *nodal* and *Pitx*, whieh relate to left-right morphological asymmetries in deuterostomes, are also present in gastropods (lophotrochozoans), and their position of expression in the developing embryo corresponds to shell coiling direction. Nevertheless, the single maternal effeets locus (see Ueshima and Asami, 2003; Schilthuizen and Davison, 2005; Davison et al., 2009) responsible for chirality in gastropods remains undiscovered. The discovery of speeimen UF 137855 provides phenotypic evidence for the first time that the sinistral allele was present in *Conus* by ~18 Ma, offering a small amount of insight into the genetie makeup of this extinct species.

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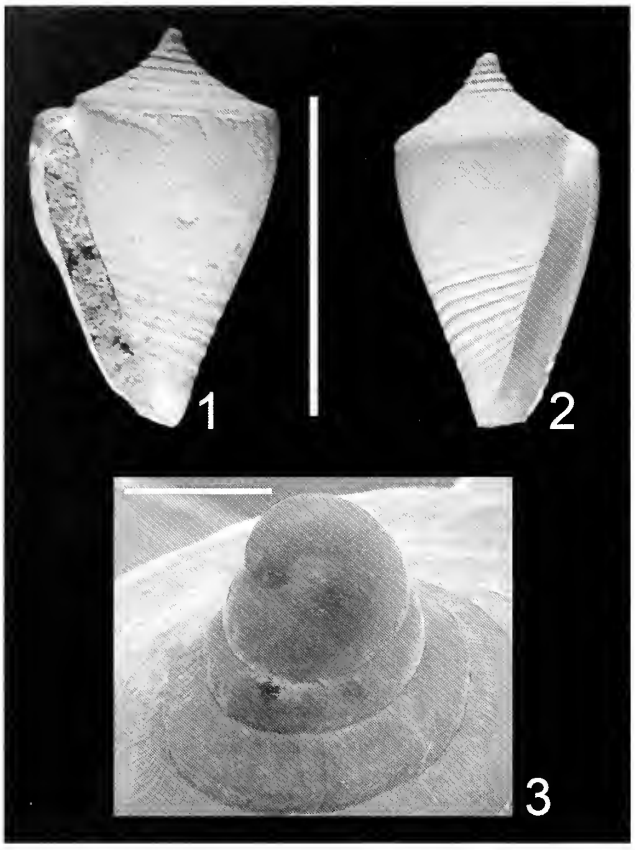

Figures 1–3. Specimens of *Conus vegrandis* Hoerle, 1976. **1.** UF 137855, sinistral specimen of *Conus* cf. *vegrandis*; shell length = 12.6 mm. **2.** UF 171658, paratype, typical dextral specimen; shell length = 11.8 mm. Both specimens are from the lower Miocene Chipola Formation of Tennile Creek, Calhoun County, Florida, USA (UF locality CA020). Scale bar (Figures 1, 2) = 1 cm. **3.** UF 173382, scanning electron micrograph of the protoconch and tuberculate early postnuclear whorls of a specimen from the lower Miocene Chipola Formation of Tennile Creek, Calhoun County, Florida, USA (UF locality CA017). Scale bar = 0.5 mm.

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Manuscripts: Each original manuscript and accompanying illustrations should be submitted to the editor preferably via e-mail or as hardcopy in triplicate.

Text must conform to the dimensions of 8½ × 11-inch paper, double-spaced, and single-column throughout (including literature cited, tables, and figure captions). Authors should follow the general recommendations of *Scientific Style and Format—The CSE Manual for Authors, Editors, and Publishers*, available from the Council of Science Editors at www.councilscienceeditors.org. The first mention of a scientific name in the text should be accompanied by the taxonomic authority, including year. Latinized names and other words to be printed in italics must be underlined; leave other formatting indications to the editor. Metric, not English, units are to be used. Geochronologic modifiers should be capitalized only when units are formally recognized: for instance, use Late Cretaceous but early Miocene. Likewise, only modifiers of formally recognized chronostratigraphic units are capitalized: use Lower Jurassic but upper Oligocene.

The sequence of sections should be title page, abstract, introduction, materials and methods, results, discussion, acknowledgments, literature cited, tables, figure captions, figures. The title page should include the title, author's name(s) and address(es). If corresponding author is not the senior author, please indicate. The abstract should summarize in 250 words or less the scope, main results, and conclusions of the article. Abstracts should be followed by a list of additional key words. All references cited in the text must appear in the Literature Cited section and vice-versa. Please follow a recent issue of THE NAUTILUS for bibliographic style, noting that journal titles must be unabbreviated. Information on plates and figures should be cited only if not included within the pagination of cited work. Tables must be numbered and each placed on a separate page. If in doubt, please follow a recent issue of the journal for sequence of sections and other style requirements.

Illustrations: Illustrations are rendered either at full-page width (maximum width 17 cm) or column width (maximum width 8.2 cm). Please take these dimensions into consideration when preparing illustrations. Page-width illustrations ideally should span the entire width of printed page (17 cm). "Tall" page-width illustrations should be avoided, square or "landscape" formats work better. Please design plates accordingly, such that there will be enough space left at the bottom of printed page for plate caption. (Digital technology has made this task much easier.)

All line drawings must be in black, clearly detailed, and completely labeled. Abbreviation definitions must be included in the caption. Line drawings must be high resolution files at at least 600 dpi (dots per inch) resolution at actual size. Standard digital formats for line drawings include .tif, .bmp, .psd, .eps, and .pdf.

Photographs may be submitted in black-and-white or color, preferably in RGB mode if in color. Standard digital formats for photographs include .tif, .psd, .jpg, or .pdf. Photographs must be high resolution files at least 300 dpi resolution at actual size.

If more than one figure is included in an illustration, all figures are to be consecutively numbered (Figures 1, 2, 3, . . . , NOT Figures 1A, 1B, 1C, . . . , NOR Plate 1, Figure 1, . . .). In illustrations with more than one figure, make sure that blank areas between figures is kept to a minimum, thereby allowing for more area for each individual figure.

Compressed files (e.g., .jpg) may be used to facilitate transmission of files during original submission, but may not be acceptable at final submission (see below).

Voucher Specimens: Deposition of the holotype in a recognized institutional, public collection is a requirement for publication of articles in which new species-level taxa are described. Deposition of paratypes in institutional collections is strongly encouraged, as is the deposition of representative voucher specimens for all other types of research work.

The Editorial Process: Upon receipt, all manuscripts are assigned a number and acknowledged. The editor reserves the right to return manuscripts that are substandard or not appropriate in scope for THE NAUTILUS. Manuscripts deemed appropriate for the journal will be sent for critical review to at least two reviewers. The reviewers' recommendations will serve as basis for rejection or continuation of the editorial process. Reviewed manuscripts will be sent back to authors for consideration of the reviewers' comments. The revised version of the manuscript may at this point be considered accepted for publication by the journal.

Final Submission: Authors of accepted manuscripts are required to submit a final version via e-mail to the editor at jleal@shellmuseum.org. Please do not send low-resolution or compressed illustration files at this stage. Send any files larger than 20 Mb on a CD or DVD to the editor.

Proofs: After typesetting, proofs will be sent to the author. Author should read proofs carefully and send corrections to the editor within 48 hours. Changes other than typesetting errors will be charged to the author at cost.

Offprints: An order form for offprints will accompany the proofs. Offprints will be ordered through the editor. Authors with institutional, grant, or other research support will be asked to pay for page charges at the rate of $60 per page.

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